

1226026

# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS, SHALL COME;

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office

*September 16, 2004*

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/478,664  
FILING DATE: *June 13, 2003*  
RELATED PCT APPLICATION NUMBER: PCT/US04/18389

Certified by



Jon W Dudas

Acting Under Secretary of Commerce  
for Intellectual Property  
and Acting Director of the U.S.  
Patent and Trademark Office

17231 U.S. PTO  
06/13/03

60478664-061303

06-16-03

PTO/SB/16 (05-03)

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE  
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 332846444US

17602 U.S. PTO  
60/478664  
06/13/03

INVENTOR(S)					
Given Name (first and middle (if any))		Family Name or Surname		Residence (City and either State or Foreign Country)	
Graeme Tawfik		SEMPLE GHARBAOUI		San Diego, California Poway, California	
Additional inventors are being named on the <u>one (1)</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Pyrazole Derivatives and Methods of Prophylaxis or Treatment of Metabolic-Related Disorders Thereof					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number 00027737 <span>→</span> Place Customer Number Bar Code Label here					
OR Type Customer Number here					
<input checked="" type="checkbox"/> Firm or Individual Name		Arena Pharmaceuticals, Inc.			
Address		IP Dept. / Legal			
Address		8166 Nancy Ridge Drive			
City		San Diego		State	CA
Country		USA		ZIP	
		Telephone	858-453-7200	Fax	858/677-0065
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		<input type="checkbox"/> CD(s); Number		<input type="checkbox"/>	
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets		<input checked="" type="checkbox"/> Other (specify)		Sequence Listing (4 pages)	
<input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.					
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees.					
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-1441					
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
FILING FEE AMOUNT (\$) 160.00					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are:					

Respectfully submitted,

[Page 1 of 2]

SIGNATURE *David L. Bradfute*  
TYPED or PRINTED NAME David L. Bradfute, Ph.D., J.D.

Date 6/3/2003

REGISTRATION NO.  
(If appropriate)  
Docket Number:

39,117

59.US1.PRO

TELEPHONE (858) 453-7200

**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

60478664 .061303

PROVISIONAL APPLICATION COVER SHEET  
Additional Page

PTO/SB/18 (05-03)  
Approved for use through 4/30/2003. OMB 0651-0032  
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE  
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Docket Number 59.US1.PRO

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle (if any))	Family or Surname	Residence (City and either State or Foreign Country)
Young-Jun	SHIN	San Diego, California
Marc	DECAIRE	San Diego, California
Claudia	AVERBUJ	San Diego, California
Philip	SKINNER	San Diego, California

[Page 2 of 2]

Number \_\_\_\_\_ of \_\_\_\_\_

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

60478664-0061303

PTO/SB/17 (10-02)

Approved for use through 10/31/2002. OMB 0651-0032  
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE  
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

# FEE TRANSMITTAL for FY 2003

Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$ 160.00

## Complete if Known

Application Number	
Filing Date	June 13, 2003
First Named Inventor	Graeme SEMPLE, et al.
Examiner Name	n/a
Art Unit	n/a
Attorney Docket No.	59.US1.PRO

## METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 50-1441  
Deposit Account Name: Arena Pharmaceuticals, Inc.

The Commissioner is authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Credit any overpayments  
☒ Charge any additional fee(s) during the pendency of this application  
☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

## FEE CALCULATION

### 1. BASIC FILING FEE

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1001 740	2001 370	Utility filing fee	
1002 330	2002 165	Design filing fee	
1003 510	2003 255	Plant filing fee	
1004 740	2004 370	Reissue filing fee	
1005 160	2005 80	Provisional filing fee	160.00
SUBTOTAL (1)			(\$ 160.00

### 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims:  - 20\*\* =  X  =   
Independent Claims:  - 3\*\* =  X  =   
Multiple Dependent:  =

<u>Large Entity</u>		<u>Small Entity</u>		<u>Fee Description</u>
Fee Code	Fee (\$)	Fee Code	Fee (\$)	
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

\*\*or number previously paid, if greater; For Reissues, see above

## FEE CALCULATION (continued)

### 3. ADDITIONAL FEES

Large Entity Small Entity

Fee Code (\$)	Fee Code (\$)	Fee Description	Fee Paid
1051 130	2051 65	Surcharge - late filing fee or oath	
1052 50	2052 25	Surcharge - late provisional filing fee or cover sheet	
1053 130	1053 130	Non-English specification	
1812 2,520	1812 2,520	For filing a request for ex parte reexamination	
1804 920*	1804 920*	Requesting publication of SIR prior to Examiner action	
1805 1,840*	1805 1,840*	Requesting publication of SIR after Examiner action	
1251 110	2251 55	Extension for reply within first month	
1252 400	2252 200	Extension for reply within second month	
1253 920	2253 460	Extension for reply within third month	
1254 1,440	2254 720	Extension for reply within fourth month	
1255 1,960	2255 980	Extension for reply within fifth month	
1401 320	2401 160	Notice of Appeal	
1402 320	2402 160	Filing a brief in support of an appeal	
1403 280	2403 140	Request for oral hearing	
1451 1,510	1451 1,510	Petition to institute a public use proceeding	
1452 110	2452 55	Petition to revive - unavoidable	
1453 1,280	2453 640	Petition to revive - unintentional	
1501 1,280	2501 640	Utility issue fee (or reissue)	
1502 460	2502 230	Design issue fee	
1503 620	2503 310	Plant issue fee	
1460 130	1460 130	Petitions to the Commissioner	
1807 50	1807 50	Processing fee under 37 CFR 1.17(q)	
1808 180	1808 180	Submission of Information Disclosure Stmt	
8021 40	8021 40	Recording each patent assignment per property (times number of properties)	
1809 740	2809 370	Filing a submission after final rejection (37 CFR 1.129(a))	
1810 740	2810 370	For each additional invention to be examined (37 CFR 1.129(b))	
1801 740	2801 370	Request for Continued Examination (RCE)	
1802 900	1802 900	Request for expedited examination of a design application	

Other fee (specify)

\*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$ 0.00

SUBMITTED BY Express Mail Label No. EV 332946444 US

Name (Print/Type)	David L. Bradfute, Ph.D., J.D.	Registration No. (Attorney/Agent)	39,117	Telephone	(858) 453-7200, ext.
Signature	<i>David L. Bradfute</i>	Date	13 June 2003		

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, Washington, DC 20231.

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

60478664-061303



Arena Pharmaceuticals, Inc.

Telephone: (858) 453-7200, x. 1254  
E-Mail: cmcclurc@arcnapharm.com

June 13, 2003

**Express Mail Label No. EV 332846444 US**

Assistant Commissioner for Patents  
Box: Patent Application  
Washington, D.C. 20231

RE: U.S. Provisional Patent Application  
For: "Pyrazole Derivatives and Methods of Prophylaxis or Treatment of Metabolic-Related Disorders Thereof"  
Inventor(s): Graeme SEMPLE, Tawfik GHARBAOUI, Young-Jun SHIN, Marc DECAIRE, Claudia AVERBUJ, Philip SKINNER  
Our Ref.: 59.US1.PRO

Dear Sir:

Enclosed please find the above-identified Provisional application for filing with the United States Patent and Trademark Office. The following documents are transmitted herewith:

1) Specification, Claims and Abstract	Quantity
2) Application Data Sheet	132 pages
3) Sequence Listing (paper copy only)	6 pages
4) Figures 1-10	4 pages
5) Provisional Application for Patent Cover Sheet;	12 sheets
Fee Transmittal Sheet for FY 2003	3 sheets
6) Authorization to charge Deposit Account 50-1441 in the amount of \$160.00 for the filing fee - (see Fee Transmittal Sheet & Application Cover Sheet)	
7) Return Receipt Postcard	

The Commissioner is hereby authorized to charge any additional fees, or credit any overpayment in the processing of these documents to our Deposit Account No. 50-1441.

Very truly yours,

ARENA PHARMACEUTICALS, INC.

David L. Bradfute, Ph.D., J.D.  
Director, Intellectual Property  
Reg. No. 39,117

DB / LS / cmn  
Enclosures

**Certificate of Mailing under 37 CFR 1.10**

Express Mail Label No.: EV 332846444 US Date of Deposit: June 13, 2003  
I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10, on the Date of Deposit shown above, postage prepaid and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Signature

Typed or Printed Name of Person Signing Certificate

6166 Nancy Ridge Drive • San Diego, CA 92121 • 858.453.7200 • Fax: 858.453.7210

## APPLICATION DATA SHEET

## Application Information

Application Number::	
Filing Date::	06 / 13 / 03
Application Type::	Provisional
Subject Matter::	Utility
Suggested Classification::	Unknown
Suggested Group Art Unit::	Unknown
CD-ROM or CD-R?::	None
Number of CD disks::	0
Number of copies of CDs::	0
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Number of Copies of CRF::	0
Title::	Pyrazole Derivatives and Methods of Prophylaxis or Treatment of Metabolic-Related Disorders Thereof
Attorney Docket Number::	59.US1.PRO
Request for Early Publication?::	N/A
Request for Non-Publication?::	N/A
Suggested Drawing Figure::	1
Total Drawing Sheets::	12
Small Entity?::	No
Petition included?::	No
Petition Type::	N/A
Licensed US Govt. Agency::	None
Contract or Grant Numbers::	None
Secrecy Order in Parent Appl.?::	No

## APPLICATION DATA SHEET

## Applicant Information

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: U.K.  
Status:: Full - Capacity  
Given Name:: Graeme  
Middle Name::  
Family Name:: SEMPLE  
Name Suffix::  
City of Residence:: San Diego  
State or Province of Residence:: California  
Country of Residence:: USA  
Street of Mailing Address:: 15920 Camino Codorniz  
City of Mailing Address:: San Diego  
State or Province of Mailing Address:: CA  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92127

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: Morocco and France  
Status:: Full - Capacity  
Given Name:: Tawfik  
Middle Name::  
Family Name:: GHARBAOUI  
Name Suffix::  
City of Residence:: Poway  
State or Province of Residence:: California  
Country of Residence:: USA  
Street of Mailing Address:: 13976 Country Creek Road

## APPLICATION DATA SHEET

City of Mailing Address:: Poway  
State or Province of Mailing Address:: CA  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92064

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: Korea  
Status:: Full - Capacity  
Given Name:: Young-Jun  
Middle Name::  
Family Name:: SHIN  
Name Suffix::  
City of Residence:: San Diego  
State or Province of Residence:: California  
Country of Residence:: USA  
Street of Mailing Address:: 11752 Westview Parkway, #3  
City of Mailing Address:: San Diego  
State or Province of Mailing Address:: CA  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92126

Applicant Authority Type:: Inventor  
Primary Citizenship Country::  
Status:: Full - Capacity  
Given Name:: Marc  
Middle Name::  
Family Name:: DECAIRE  
Name Suffix::

## APPLICATION DATA SHEET

City of Residence:: San Diego  
State or Province of Residence:: California  
Country of Residence:: USA  
Street of Mailing Address:: 10504 Caminito Alvarez  
City of Mailing Address:: San Diego  
State or Province of Mailing Address:: CA  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92126

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: Israel and Argentina  
Status:: Full - Capacity  
Given Name:: Claudia  
Middle Name::  
Family Name:: AVERBUJ  
Name Suffix::  
City of Residence:: San Diego  
State or Province of Residence:: California  
Country of Residence:: USA  
Street of Mailing Address:: 4110 Via Candidz #103  
City of Mailing Address:: San Diego  
State or Province of Mailing Address:: CA  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92130

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: United Kingdom  
Status:: Unknown

## APPLICATION DATA SHEET

Given Name:: Philip  
Middle Name:: J.  
Family Name:: SKINNER  
Name Suffix::  
City of Residence:: San Diego  
State or Province of Residence:: California  
Country of Residence:: USA  
Street of Mailing Address:: 1222 Oliver Avenue, #2  
City of Mailing Address:: San Diego  
State or Province of Mailing Address:: CA  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92109

## Correspondence Information

Name:: C. M. McClure  
ARENA PHARMACEUTICALS, INC.  
Street of Mailing Address:: 6166 Nancy Ridge Drive  
City of Mailing Address:: San Diego  
State or Province of Mailing Address:: CA  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92121  
Telephone Number One:: (858) 453-7200  
Telephone Number Two::  
Fax Number:: (858) 677-0065  
E-Mail Address:: cmcclure@arenapharm.com

60478664-061303

## APPLICATION DATA SHEET

### Representative Information

Representative Customer Number:: 000027737

Representative Designation::	Registration Number::	Representative Name::
Primary	39,117	David L. Bradfute
Associate	46,087	Ann A. Nguyen
Associate	50,711	Deno P. Dialynas
Associate	53,631	Lyle W. Spruce

Assignee Name:: **Arena Pharmaceuticals, Inc.**  
Street of Mailing Address:: 6166 Nancy Ridge Drive  
City of Mailing Address:: San Diego  
State of Mailing Address:: California  
Country of Mailing Address:: USA  
Postal or Zip Code of Mailing Address:: 92121

Express Mail Label No. EV 332846444 US

59.US1.PRO

Provisional Application

**PYRAZOLE DERIVATIVES AND METHODS OF PROPHYLAXIS OR  
TREATMENT OF METABOLIC-RELATED DISORDERS THEREOF**

5

**FIELD OF THE INVENTION**

The present invention relates to certain pyrazole carboxylic acid and ester derivatives, and pharmaceutically acceptable salts thereof, which exhibit useful pharmaceutical properties, for example as agonists for the nicotinic acid receptor, RUP25. Also provided by the present invention are pharmaceutical compositions containing one or more compounds of the invention, and methods of using the compounds and compositions of the invention in the prophylaxis or treatment of metabolic-related disorders, including dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance, type 2 diabetes, Syndrome-X and the like. In addition, the present invention also provides for the use of the compounds of the invention in combination with other active agents such as those belonging to the class of  $\alpha$ -glucosidase inhibitors, aldose reductase inhibitors, biguanides, HMG-CoA reductase inhibitors, squalene synthesis inhibitors, fibrates, LDL catabolism enhancers, angiotensin converting enzyme (ACE) inhibitors, insulin secretion enhancers, thiazolidinedione and the like.

20

**BACKGROUND OF THE INVENTION**

**Compounds of the invention as Antilipolytic Agents**

Atherosclerosis and stroke are the numbers one and number three leading causes of death of both men and women in the United States. Type 2 diabetes is a public health problem that is serious, widespread and increasing. Elevated levels of low density lipoprotein (LDL) cholesterol or low levels of high density lipoprotein (HDL) cholesterol are, independently, risk factors for atherosclerosis and associated cardiovascular pathologies. In addition, high levels of plasma free fatty acids are associated with insulin resistance and type 2 diabetes. One strategy for decreasing LDL-cholesterol, increasing HDL-cholesterol, and decreasing plasma free fatty acids is to inhibit lipolysis in adipose tissue. This approach involves regulation of hormone sensitive lipase, which is the rate-limiting enzyme in lipolysis. Lipolytic agents increase cellular levels of cAMP, which leads to activation of hormone sensitive lipase within

59.US1.PRO

## Provisional Application

adipocytes. Agents that lower intracellular cAMP levels, by contrast, would be antilipolytic.

It is also worth noting in passing that an increase in cellular levels of cAMP down-regulates the secretion of adiponectin from adipocytes [Delporte, ML et al. *Biochem J* (2002) July]. Reduced levels of plasma adiponectin have been associated with metabolic-related disorders, including atherosclerosis, coronary heart disease, insulin resistance and type 2 diabetes [Matsuda, M et al. *J Biol Chem* (2002) July and reviewed therein].

Nicotinic acid (niacin, pyridine-3-carboxylic acid) is a water-soluble vitamin required by the human body for health, growth and reproduction; a part of the Vitamin B complex. Nicotinic acid is also one of the oldest used drugs for the treatment of dyslipidemia. It is a valuable drug in that it favorably affects virtually all of the lipid parameters listed above [Goodman and Gilman's Pharmacological Basis of Therapeutics, editors Harmon JG and Limbird LE, Chapter 36, Mahley RW and Bersot TP (2001) pages 971-1002]. The benefits of nicotinic acid in the treatment or prevention of atherosclerotic cardiovascular disease have been documented in six major clinical trials [Guyton JR (1998) *Am J Cardiol* 82:18U-23U]. Nicotinic acid and related derivatives, such as, acipimox have recently been discussed [Lorenzen, A et al (2001) *Molecular Pharmacology* 59:349-357]. Structure and synthesis of additional analogs or derivatives of nicotinic acid are discussed throughout the Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals, Tenth Edition (1983), which is incorporated herein by reference in its entirety.

Nicotinic acid and currently existing analogs thereof inhibit the production and release of free fatty acids from adipose tissue, likely via an inhibition of adenylyl cyclase, a decrease in intracellular cAMP levels, and a concomitant decrease in hormone sensitive lipase activity. Agonists that down-regulate hormone sensitive lipase activity leading to a decrease in plasma free fatty acid levels are likely to have therapeutic value. The consequence of decreasing plasma free fatty acids is two-fold. First, it will ultimately lower LDL-cholesterol and raise HDL-cholesterol levels, independent risk factors, thereby reducing the risk of mortality due to cardiovascular incidence subsequent to atheroma formation. Second, it will provide an increase in insulin sensitivity in individuals with insulin resistance or type 2 diabetes. Unfortunately, the use of nicotinic acid as a therapeutic is partially limited by a number of associated, adverse side-effects. These include flushing, free fatty acid rebound, and liver toxicity.

59.US1.PRO

Provisional Application

The rational development of novel, nicotinic acid receptor agonists that have fewer side-effects will be valuable, but to date this has been hindered by the inability to molecularly identify the nicotinic acid receptor. Furthermore, other receptors of the same class may exist on the surface of adipocytes and similarly decrease hormone sensitive lipase activity through a reduction in the level of intracellular cAMP but without the elicitation of adverse effects such as flushing, thereby representing promising novel therapeutic targets. Recent work suggests that nicotinic acid probably acts through a specific GPCR [Lorenzen A, et al. (2001) Molecular Pharmacology 59:349-357 and reviewed therein]. Further work has suggested that the effects of nicotinic acid on macrophages, spleen and probably adipocytes are mediated via this specific GPCR [Lorenzen A, et al. (2002) Biochemical Pharmacology 64:645-648 and reviewed therein].

**G Protein-Coupled Receptors**

Although a number of receptor classes exist in humans, by far the most abundant and therapeutically relevant is represented by the G protein-coupled receptor (GPCR) class. It is estimated that there are some 30,000-35,000 genes within the human genome, and of these, approximately 2% are estimated to code for GPCRs. Receptors, including GPCRs, for which the endogenous ligand has been identified, are referred to as "known" receptors, while receptors for which the endogenous ligand has not been identified are referred to as "orphan" receptors.

GPCRs represent an important area for the development of pharmaceutical products: from approximately 20 of the 100 known GPCRs, approximately 60% of all prescription pharmaceuticals have been developed. For example, in 1999, of the top 100 brand name prescription drugs, the following drugs interact with GPCRs (the primary diseases and/or disorders treated related to the drug is indicated in parentheses):

Claritin® (allergies)	Prozac® (depression)	Vasotec® (hypertension)
Paxil® (depression)	Zoloft® (depression)	Zyprexa® (psychotic disorder)
Cozaar® (hypertension)	Imitrex® (migraine)	Zantac® (reflux)
Propulsid® (reflux disease)	Risperdal® (schizophrenia)	Serevent® (asthma)
Pepcid® (reflux)	Gaster® (ulcers)	Atrovent® (bronchospasm)
Effexor® (depression)	Depakote® (epilepsy)	Cardura® (prostatic hypertrophy)
Allegra® (allergies)	Lupron® (prostate cancer)	Zoladex® (prostate cancer)
Diprivan® (anesthesia)	BuSpar® (anxiety)	Ventolin® (bronchospasm)
Hytrin® (hypertension)	Wellbutrin® (depression)	Zyrtec® (rhinitis)

59.US1.PRO

## Provisional Application

Plavix® (MI/stroke)      Toprol-XL® (hypertension)      Tenormin® (angina)  
 Xalatan® (glaucoma)      Singulair® (asthma)      Diovan® (hypertension)  
 Hamal® (prostatic hyperplasia)  
 (Med Ad News 1999 Data).

5            GPCRs share a common structural motif, having seven sequences of between 22  
 to 24 hydrophobic amino acids that form seven alpha helices, each of which spans the  
 membrane (each span is identified by number, *i.e.*, transmembrane-1 (TM-1),  
 transmembrane-2 (TM-2), *etc.*). The transmembrane helices are joined by strands of  
 10 amino acids between transmembrane-2 and transmembrane-3, transmembrane-4 and  
 transmembrane-5, and transmembrane-6 and transmembrane-7 on the exterior, or  
 "extracellular" side, of the cell membrane (these are referred to as "extracellular" regions  
 1, 2 and 3 (EC-1, EC-2 and EC-3), respectively). The transmembrane helices are also  
 joined by strands of amino acids between transmembrane-1 and transmembrane-2,  
 transmembrane-3 and transmembrane-4, and transmembrane-5 and transmembrane-6 on  
 15 the interior, or "intracellular" side, of the cell membrane (these are referred to as  
 "intracellular" regions 1, 2 and 3 (IC-1, IC-2 and IC-3), respectively). The "carboxy"  
 ("C") terminus of the receptor lies in the intracellular space within the cell, and the  
 "amino" ("N") terminus of the receptor lies in the extracellular space outside of the cell.

20            Generally, when a ligand binds with the receptor (often referred to as  
 "activation" of the receptor), there is a change in the conformation of the receptor that  
 facilitates coupling between the intracellular region and an intracellular "G-protein." It  
 has been reported that GPCRs are "promiscuous" with respect to G proteins, *i.e.*, that a  
 GPCR can interact with more than one G protein. *See*, Kenakin, T., 43 *Life Sciences*  
 1095 (1988). Although other G proteins exist, currently, Gq, Gs, Gi, Gz and Go are G  
 25 proteins that have been identified. Ligand-activated GPCR coupling with the G-protein  
 initiates a signaling cascade process (referred to as "signal transduction"). Under normal  
 conditions, signal transduction ultimately results in cellular activation or cellular  
 inhibition. Although not wishing to be bound to theory, it is thought that the IC-3 loop  
 as well as the carboxy terminus of the receptor interact with the G protein.

30            Gi-coupled GPCRs lower intracellular cAMP levels. The Melanophore  
 technology (see *infra*) is useful for identifying Gi-coupled GPCRs.

          Under physiological conditions, GPCRs exist in the cell membrane in  
 equilibrium between two different conformations: an "inactive" state and an "active"  
 state. A receptor in an inactive state is unable to link to the intracellular signaling

59.US1.PRO

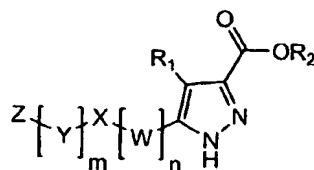
## Provisional Application

transduction pathway to initiate signal transduction leading to a biological response. Changing the receptor conformation to the active state allows linkage to the transduction pathway (via the G-protein) and produces a biological response.

A receptor can be stabilized in an active state by a ligand or a compound such as a drug. Recent discoveries, including but not exclusively limited to modifications to the amino acid sequence of the receptor, provide means other than ligands or drugs to promote and stabilize the receptor in the active state conformation. These means effectively stabilize the receptor in an active state by simulating the effect of a ligand binding to the receptor. Stabilization by such ligand-independent means is termed "constitutive receptor activation."

## SUMMARY OF THE INVENTION

One aspect of the present invention encompasses pyrazole carboxylic acid and ester derivatives as shown in Formula (I):



(I)

wherein:

W and Y are independently a straight or branched chain  $C_{1-5}$  alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said  $C_{1-5}$  alkylene group is optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  haloalkyl or  $C_{1-4}$  alkoxy;

X is  $-NR_3C(O)-$ ,  $-C(O)NR_3$ ,  $-NR_3S(O)_2-$ ,  $-S(O)_2NR_3-$ ,  $-NR_3C(O)NR_4-$ ,  $-NR_3C(O)O-$ ,  $-OC(O)NR_3-$ ,  $-NR_3-$ ,  $-C(O)-$ ,  $-CH(OH)-$ ,  $-C(NH)-$ ,  $-O-$ ,  $-S-$ ,  $-S(O)-$  or  $-S(O)_2-$ ;

$R_3$  and  $R_4$  are independently H,  $C_{1-4}$  alkyl, phenyl or heteroaryl, wherein each of said alkyl, phenyl or heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxyl, thiol, cyano, nitro,  $C_{1-4}$  haloalkyl, amino,  $C_{1-4}$  aminoalkyl,  $C_{2-4}$  aminodialkyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  alkoxy,  $C_{2-4}$  alkenyl,  $C_{2-4}$  alkynyl,  $C_{1-4}$  haloalkoxy,  $C_{1-4}$  alkylthio,  $C_{1-4}$  alkylsulfinyl,  $C_{1-4}$  alkylsulfonyl,  $C_{1-4}$  haloalkylthio,  $C_{1-4}$  haloalkylsulfinyl and  $C_{1-4}$  haloalkylsulfonyl;

## 59.US1.PRO

## Provisional Application

Z is H, halogen, phenyl or heteroaryl, wherein said phenyl and heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxy, thiol, cyano, nitro, C<sub>1-4</sub> haloalkyl, amino, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

R<sub>1</sub> is H, hydroxyl, halogen, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> haloalkyl;

R<sub>2</sub> is H or C<sub>1-8</sub> alkyl and

"n" and "m" or independently 0 or 1; or

a pharmaceutically acceptable salt, solvate or hydrate thereof; provided that:

i) when both R<sub>1</sub> and R<sub>2</sub> are H then -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not CO<sub>2</sub>H, C(O)-C<sub>6</sub>H<sub>4</sub>-p-O-C<sub>8</sub>H<sub>17</sub>, OCH<sub>2</sub>CH<sub>3</sub>, OH, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H, CH<sub>2</sub>CO<sub>2</sub>H or CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H;

ii) when R<sub>1</sub> is CH<sub>3</sub> and R<sub>2</sub> is H then -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not CH<sub>2</sub>CO<sub>2</sub>H, C(O)CH-CH C<sub>6</sub>H<sub>5</sub>, C(O)C<sub>6</sub>H<sub>4</sub>-p-OCH<sub>3</sub>, CO<sub>2</sub>H, C(O)CH<sub>3</sub>, C(O)C<sub>6</sub>H<sub>4</sub>-o-CH<sub>3</sub>, C(O)C<sub>6</sub>H<sub>4</sub>-o-Br, C(O)C<sub>6</sub>H<sub>4</sub>-o-Cl, or C(O)C<sub>6</sub>H<sub>5</sub>;

iii) when R<sub>1</sub> is Br and R<sub>2</sub> is H then -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not CO<sub>2</sub>H;

iv) when R<sub>1</sub> is OH and R<sub>2</sub> is H then -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not CO<sub>2</sub>H;

v) when R<sub>1</sub> is H and R<sub>2</sub> is CH<sub>3</sub> then -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not 2,6-dichloro-4-trifluoromethylphenoxy, C(O)NH-C<sub>6</sub>H<sub>4</sub>-p-OCH<sub>2</sub>CH<sub>3</sub>, NHC(O)CH(CH<sub>3</sub>)<sub>2</sub>, SCH<sub>3</sub>, C(O)-C<sub>6</sub>H<sub>4</sub>-p-O-C<sub>8</sub>H<sub>17</sub>, SCH<sub>2</sub>CH<sub>3</sub>, C(O)NHC<sub>6</sub>H<sub>5</sub>, CH(OCH<sub>3</sub>)<sub>2</sub>, CH<sub>2</sub>OC(O)CH<sub>3</sub>, CO<sub>2</sub>H, CO<sub>2</sub>CH<sub>3</sub>, C(O)C<sub>6</sub>H<sub>4</sub>-p-NO<sub>2</sub>, C(O)C<sub>6</sub>H<sub>5</sub>, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> or CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>;

vi) when R<sub>1</sub> is OH and R<sub>2</sub> is CH<sub>3</sub> then -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not CH<sub>2</sub>OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, CH<sub>2</sub>OCH(CH<sub>3</sub>)<sub>2</sub> or CH<sub>2</sub>OH;

vii) when R<sub>2</sub> is CH<sub>3</sub> then:

R<sub>1</sub> is not CH<sub>3</sub> and -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not 2,6-dichloro-4-trifluoromethylphenoxy;

R<sub>1</sub> is not I and -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is not CO<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>;

59.US1.PRO

## Provisional Application

$R_1$  is not  $C(CH_3)_3$  and  $-[W]_n-X-[Y]_m-Z$  together is not formyl;

$R_1$  is not Br and  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2CH_3$ ;

or

$R_1$  is not  $CH_2CH_2CH_2CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is not formyl;

viii) when  $R_1$  is H and  $R_2$  is  $CH_2CH_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not  $CH_2SCH_2CH_3$ ,  $OCH_2CH_2CH=CH_2$ ,  $CH_2CH_2CH_2OH$ ,  $CH_2CH_2CHO$ ,  $CO_2CH_2CH_3$ ,  $OCH_3$ ,  $C(O)CH_2Br$ ,  $CO_2C_8H_{17}$ , formyl, OH,  $CH_2N(CH_2CH_2Cl)_2$ ,  $CH(CH_3)OC(O)CH_3$ ,  $CH_2OH$ ,  $CH_2OC(O)CH_3$ ,  $C(O)CH_3$ ,  $C(O)C_6H_5$  or  $C(O)NHCH_2CO_2CH_2CH_3$ .

ix) when  $R_1$  is  $CH_3$  and  $R_2$  is  $CH_2CH_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not  $CH(OH)C_6H_4-p-N(CH_3)_2$ ,  $C(O)CH_2C(O)CH_3$ ,  $CO_2CH_2C_6H_5$ ,  $CO_2CH_3$ ,  $C(O)CH_2CH_2CH_3$ ,  $C(O)CH_3$ ,  $C(O)C_6H_4-p-OCH_3$ ,  $C(O)C_6H_4-o-Br$ ,  $C(O)C_6H_4-p-Cl$ ,  $C(O)C_6H_4-o-Cl$ ,  $C(O)CH_2C_6H_5$  or  $C(O)C_6H_5$ ;

x) when  $R_2$  is  $CH_2CH_3$  then:

$R_1$  is not I and  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2CH_2CH_3$ ;

$R_1$  is not  $CF_3$  and  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2CH_2CH_3$ ; or

$R_1$  is not Br and  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2CH_2CH_3$ ;

xi) when  $R_1$  is OH and  $R_2$  is  $CH_2CH_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not  $C(O)C_6H_5$ ,  $C(O)NH_2$  or  $CO_2CH_2CH_3$ ;

xii) when  $R_1$  is H and  $R_2$  is  $C(CH_3)_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2C(CH_3)_3$ ,  $C(O)NHC(O)CH_3$  or  $C(O)NH_2$ ;

xiii) when  $R_1$  is OH and  $R_2$  is  $CH_2CH_2CH_2CH_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not  $C(O)C_6H_5$ ; or

xiv) when X is  $-NR_3-$  then "n" is not 0.

One embodiment of the present invention pertains to compounds of Formula (I) wherein "n" is 0.

One embodiment of the present invention pertains to compounds of Formula (I) wherein "n" is 1.

59.US1.PRO

## Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein "m" is 0.

One embodiment of the present invention pertains to compounds of Formula (I) wherein "m" is 1.

5 One embodiment of the present invention pertains to compounds of Formula (I) wherein W is the straight or branched chain  $C_{1-5}$  alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said  $C_{1-5}$  alkylene group is optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy.

10 One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-CH_2-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-CH(CH_3)-$  optionally substituted with halogen, hydroxyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-C(CH_3)_2-$ .

15 One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-CH_2CH_2-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-CH(CH_3)CH_2-$  or  $-CH_2CH(CH_3)-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-C(CH_3)_2CH_2-$  or  $-CH_2C(CH_3)_2-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-CH(OCH_3)CH_2-$  or  $-CH_2CH(OCH_3)-$  optionally substituted with halogen, hydroxyl or  $C_{1-4}$  alkyl.

20 One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-CH_2CH_2CH_2-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy.

25 One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-CH_2CH_2CH_2CH_2-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-CH=CH-$  optionally substituted with  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-C\equiv C-$ .

30 One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-C(O)-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-CH_2C(O)-$  or  $-C(O)CH_2-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-CH(CH_3)C(O)-$  or -

59.US1.PRO

## Provisional Application

$\text{C(O)CH(CH}_3\text{)}$ - optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{C(CH}_3\text{)}_2\text{C(O)-}$  or  $-\text{C(O)C(CH}_3\text{)}_2-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{CH}_2\text{C(O)-}$  or  $-\text{C(O)CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{C(CH}_3\text{)}_2\text{CH}_2\text{C(O)-}$  or  $-\text{C(O)CH}_2\text{C(CH}_3\text{)}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{C(O)CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)-}$  or  $-\text{C(O)CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH(CH}_3\text{)CH}_2\text{CH}_2\text{C(O)-}$  or  $-\text{C(O)CH}_2\text{CH}_2\text{CH(CH}_3\text{)-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{CH}_2\text{C(O)CH}_2-$  or  $-\text{CH}_2\text{C(O)CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH=CHC(O)-}$  or  $-\text{C(O)CH=CH-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{C(CH}_3\text{)=CHC(O)-}$  or  $-\text{C(O)CH=C(CH}_3\text{)-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is the straight or branched chain  $\text{C}_{1-5}$  alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said  $\text{C}_{1-5}$  alkylene group is optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH(CH}_3\text{)-}$  optionally substituted with halogen, hydroxyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{C(CH}_3\text{)}_2-$ .

59.US1.PRO

## Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}(\text{CH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{C}(\text{CH}_3)_2\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{CH}_3)_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}(\text{OCH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{OCH}_3)-$  optionally substituted with halogen, hydroxyl or  $\text{C}_{1-4}$  alkyl.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}=\text{CH}-$  optionally substituted with  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{C}\equiv\text{C}-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{C}\equiv\text{CCH}_2-$  or  $-\text{CH}_2\text{C}\equiv\text{C}-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{C}(\text{O})-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}(\text{CH}_3)\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{C}(\text{CH}_3)_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{C}(\text{CH}_3)_2-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{C}(\text{CH}_3)_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{C}(\text{CH}_3)_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

59.US1.PRO

## Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

5 One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

10 One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}=\text{CHC}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}=\text{CH}-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

15 One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{C}(\text{ClI}_3)=\text{CHC}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}=\text{C}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

20 One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3\text{C}(\text{O})-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{C}(\text{O})\text{NR}_3-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3\text{S}(\text{O})_2-$ .

25 One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{S}(\text{O})_2\text{NR}_3-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3\text{C}(\text{O})\text{NR}_4-$ .

30 One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3\text{C}(\text{O})\text{O}-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{OC}(\text{O})\text{NR}_3-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3-$ .

## 59.US1.PRO

## Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein  $R_3$  is H or  $CH_3$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein  $R_4$  is H or  $CH_3$ .

5 One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-C(O)-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-CH(OH)-$ .

10 One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-C(NH)-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-O-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-S-$ .

15 One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-S(O)-$ .

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-S(O)_2-$ .

20 One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is H.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is halogen.

25 One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is phenyl. In some embodiments the phenyl is optionally substituted with 1 to 5 substituents selected from the group consisting of halogen,  $C_{1-4}$  haloalkyl,  $C_{1-4}$  aminoalkyl,  $C_{2-4}$  aminodialkyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  alkoxy,  $C_{1-4}$  haloalkoxy,  $C_{1-4}$  alkylthio,  $C_{1-4}$  alkylsulfinyl,  $C_{1-4}$  alkylsulfonyl,  $C_{1-4}$  haloalkylthio,  $C_{1-4}$  haloalkylsulfinyl and  $C_{1-4}$  haloalkylsulfonyl. In some embodiments the phenyl is optionally substituted with 1 to 3 substituents selected from the group consisting of  $-F$ ,  $-Cl$ ,  $-Br$ ,  $-CF_3$ ,  $-NHCH_3$ ,  $-N(CH_3)_2$ ,  $-CH_3$ ,  $-CH_2CH_3$ ,  $-OCH_3$  and  $-OCF_3$ .

30 One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is heteroaryl. In some embodiments the heteroaryl is optionally substituted with 1 to 5 substituents selected from the group consisting of halogen,  $C_{1-4}$  haloalkyl,  $C_{1-4}$  aminoalkyl,  $C_{2-4}$  aminodialkyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  alkoxy,  $C_{1-4}$  haloalkoxy,  $C_{1-4}$  alkylthio,

59.US1.PRO

## Provisional Application

C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl. In some embodiments the phenyl is optionally substituted with 1 to 3 substituents selected from the group consisting of -F, -Cl, -Br, -CF<sub>3</sub>, -NHCH<sub>3</sub>, -N(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, -OCH<sub>3</sub> and -OCF<sub>3</sub>.

5 One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is H.

One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is hydroxyl.

10 One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is halogen. In some embodiments, R<sub>1</sub> is F, Cl or Br. In still further embodiments, R<sub>1</sub> is F.

One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is C<sub>1-4</sub> alkyl.

15 One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is C<sub>1-4</sub> haloalkyl.

One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>2</sub> is H.

One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>2</sub> is C<sub>1-8</sub> alkyl.

20 In some embodiments, compounds of the present invention are members of the group consisting of:

25 5-Ethylsulfanylmethyl-1H-pyrazole-3-carboxylic acid; 5-Ethanesulfinylmethyl-1H-pyrazole-3-carboxylic acid; 5-Ethanesulfonylmethyl-1H-pyrazole-3-carboxylic acid; 5-(2-Oxo-propoxymethyl)-1H-pyrazole-3-carboxylic acid; 5-Prop-2-ynyloxymethyl-1H-pyrazole-3-carboxylic acid; 5-Carbamoyl-1H-pyrazole-3-carboxylic acid; 5-(1-Methylsulfanyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Methanesulfinyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Methanesulfonyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Carboxy-1,1-dimethyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Acetoxy-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Hydroxy-propyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Chloro-3-hydroxy-propyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Hydroxy-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Hydroxy-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Carboxy-1-methyl-vinyl)-1H-pyrazole-3-carboxylic acid; 5-Propylcarbamoylmethyl-1H-pyrazole-3-carboxylic acid; 5-(2-Amino-vinyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Amino-propyl)-1H-pyrazole-3-carboxylic acid; 5-(2-

59.US1.PRO

## Provisional Application

Dimethylamino-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Hydroxy-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Hydroxy-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Hydroxy-2-methyl-propyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Carboxy-1-methyl-propyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Carboxy-vinyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Methoxy-vinyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Acetoxy-propyl)-1H-pyrazole-3-carboxylic acid; 5-Carbamoylmethyl-1H-pyrazole-3-carboxylic acid and 5-Hydroxymethyl-1H-pyrazole-3-carboxylic acid.

In some embodiments, compounds of the present invention are members of the group consisting of:

5-(2,2-Dimethoxy-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Imino-propyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Amino-2-methyl-propyl)-1H-pyrazole-3-carboxylic acid; 5-(Ethoxycarbonyl-fluoro-methyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Ethoxycarbonyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-Ethoxycarbonylmethyl-1H-pyrazole-3-carboxylic acid; 5-(2-Ethoxycarbonyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-Methoxymethyl-1H-pyrazole-3-carboxylic acid; 5-(1-Methoxycarbonyl-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(1-Hydroxy-1-methoxycarbonyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Methoxycarbonyl-propyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Methoxycarbonyl-vinyl)-1H-pyrazole-3-carboxylic acid; 5-Dimethylcarbamoylmethyl-1H-pyrazole-3-carboxylic acid; 1H-Pyrazole-3,5-dicarboxylic acid; 5-Ethoxymethyl-1H-pyrazole-3-carboxylic acid; 5-(2-Methoxy-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Methoxy-propyl)-1H-pyrazole-3-carboxylic acid; 5-Methylsulfanylmethyl-1H-pyrazole-3-carboxylic acid; 5-Methanesulfonylmethyl-1H-pyrazole-3-carboxylic acid; 5-(2-Methylsulfanyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Methanesulfonyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Methanesulfonyl-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Methylsulfanyl-propyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Methanesulfonyl-propyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Amino-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Methylamino-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Dimethylamino-ethyl)-1H-pyrazole-3-carboxylic acid; 5-(2-Oxo-propyl)-1H-pyrazole-3-carboxylic acid; 5-(3-Oxo-butyl)-1H-pyrazole-3-carboxylic acid and 5-(Benzylamino-methyl)-1H-pyrazole-3-carboxylic acid.

One aspect of the present invention encompasses pharmaceutical compositions comprising at least one compound according to Formula (I), as described herein. In

59.US1.PRO

Provisional Application

some embodiments, the pharmaceutical composition further comprises one or more agents selected from the group consisting of  $\alpha$ -glucosidase inhibitor, aldose reductase inhibitor, biguanide, HMG-CoA reductase inhibitor, squalene synthesis inhibitor, fibrate, LDL catabolism enhancer, angiotensin converting enzyme inhibitor, insulin secretion enhancer and thiazolidinedione.

One embodiment of the present invention pertains to pharmaceutical compositions comprising a  $\alpha$ -glucosidase inhibitor. In some embodiments the  $\alpha$ -glucosidase inhibitor is acarbose, voglibose or miglitol. In some embodiments the  $\alpha$ -glucosidase inhibitor is voglibose.

One embodiment of the present invention pertains to pharmaceutical compositions comprising an aldose reductase inhibitor. In some embodiments the aldose reductase inhibitor is tolurestat; epalrestat; imirestat; zenarestat; zopolrestat; or sorbinil.

One embodiment of the present invention pertains to pharmaceutical compositions comprising a biguanide. In some embodiments the biguanide is phenformin, metformin or buformin. In some embodiments the biguanide is metformin.

One embodiment of the present invention pertains to pharmaceutical compositions comprising a HMG-CoA reductase inhibitor. In some embodiments the HMG-CoA reductase inhibitor is rosuvastatin, pravastatin, simvastatin, lovastatin, atorvastatin, fluvastatin or cerivastatin.

One embodiment of the present invention pertains to pharmaceutical compositions comprising a fibrate. In some embodiments the fibrate is bezafibrate, beclobate, binifibrate, ciprofibrate, clinofibrate, clofibrate, clofibric acid, etofibrate, fenofibrate, gemfibrozil, nicofibrate, pirifibrate, ronifibrate, simfibrate, or theofibrate.

One embodiment of the present invention pertains to pharmaceutical compositions comprising an angiotensin converting enzyme inhibitor. In some embodiments the angiotensin converting enzyme inhibitor is captopril, enalapril, alacepril, delapril; ramipril, lisinopril, imidapril, benazepril, ceronapril, cilazapril, enalaprilat, fosinopril, moxetopril, perindopril, quinapril, spirapril, temocapril or trandolapril.

One embodiment of the present invention pertains to pharmaceutical compositions comprising an insulin secretion enhancer. In some embodiments the insulin secretion enhancer is tolbutamide; chlorpropamide; tolazamide; acetohexamide; glycopyramide; glibenclamide; gliclazide; 1-butyl-3-metanilylurea; carbutamide;

59.US1.PRO

## Provisional Application

glibonuride; glipizide; gliquidone; glisoxepid; glybuthiazole; glibuzole; glyhexamide; glymidine; glypinamide; phenbutamide; tolcyclamide, glimepiride, nateglinide, or mitiglinide.

5 One embodiment of the present invention pertains to pharmaceutical compositions comprising a thiazolidinedione. In some embodiments the thiazolidinedione is rosiglitazone or pioglitazone. In some embodiments the thiazolidinedione is rosiglitazone.

10 One aspect of the present invention pertains to a compound of Formula (I), as described herein, for use in a method of treatment of the human or animal body by therapy.

One aspect of the present invention pertains to a compound of Formula (I), as described herein, for use in a method of prophylaxis or treatment of a metabolic-related disorder of the human or animal body by therapy.

15 One aspect of the present invention pertains to methods for prophylaxis or treatment of a metabolic-related disorder in an individual in need of prophylaxis or treatment comprising administering to the individual a therapeutically effective amount of a compound according of Formula (I), as described, or a pharmaceutical composition.

20 One aspect of the present invention pertains to methods of modulating a RUP25 receptor in an individual comprising contacting the receptor with a compound according Formula (I). In some embodiments the compound is an agonist. In some embodiments the modulation of the RUP25 receptor is for prophylaxis or treatment of a metabolic-related disorder in an individual in need of said prophylaxis or treatment.

25 One embodiment of the present invention relates to methods of prophylaxis or treatment of metabolic-related disorders. In some embodiments the metabolic-related disorder is of the group consisting of dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance, obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, heart disease and type 2 diabetes. In some  
30 embodiments the metabolic-related disorder is dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance and type 2 diabetes. In some embodiments the metabolic-related disorder is dyslipidemia. In some embodiments the metabolic-related disorder is atherosclerosis. In some embodiments the metabolic-related disorder is coronary heart disease. In some embodiments the metabolic-related disorder is insulin resistance. In some embodiments the metabolic-related disorder is type 2 diabetes.

59.US1.PRO

## Provisional Application

One aspect of the present invention encompasses compounds of Formula (I) for production of a medicament for use in prophylaxis or treatment of a metabolic-related disorder. In some embodiments, the use of a compound of Formula (I) for production of a medicament further comprises one or more agents selected from the group consisting of  $\alpha$ -glucosidase inhibitor, aldose reductase inhibitor, biguanide, HMG-CoA reductase inhibitor, squalene synthesis inhibitor, fibrate, LDL catabolism enhancer, angiotensin converting enzyme inhibitor, insulin secretion enhancer and thiazolidinedione. In some embodiments the agent is a  $\alpha$ -glucosidase inhibitor. In some embodiments the  $\alpha$ -glucosidase inhibitor is acarbose, voglibose or miglitol. In some embodiments the  $\alpha$ -glucosidase inhibitor is voglibose. In some embodiments the agent is an aldose reductase inhibitor. In some embodiments the aldose reductase inhibitor is tolurestat; epalrestat; imirestat; zenarestat; zopolrestat; or sorbinil. In some embodiments the agent is a biguanide. In some embodiments the biguanide is phenformin, metformin or buformin. In some embodiments the biguanide is metformin. In some embodiments the agent is a HMG-CoA reductase inhibitor. In some embodiments the HMG-CoA reductase inhibitor is rosuvastatin, pravastatin, simvastatin, lovastatin, atorvastatin, fluvastatin or cerivastatin. In some embodiments the agent is a fibrate. In some embodiments the fibrate is bezafibrate, bezafibrate, binifibrate, ciprofibrate, clonofibrate, clofibrate, clofibric acid, etofibrate, fenofibrate, gemfibrozil, nicofibrate, pirifibrate, ronifibrate, simfibrate, or theofibrate. In some embodiments the agent is an angiotensin converting enzyme inhibitor. In some embodiments the angiotensin converting enzyme inhibitor is captopril, enalapril, alacepril, delapril; ramipril, lisinopril, imidapril, benazepril, ceronapril, cilazapril, enalaprilat, fosinopril, moxeltopril, perindopril, quinapril, spirapril, temocapril or trandolapril. In some embodiments the agent is an insulin secretion enhancer. In some embodiments the insulin secretion enhancer is tolbutamide; chlorpropamide; tolazamide; acetohexamide; glycopyramide; glibenclamide; gliclazide; 1-butyl-3-metanyllurea; carbutamide; glibonuride; glipizide; gliquidone; glisoxepid; glybuthiazole; glibuzole; glyhexamide; glymidine; glypinamide; phenbutamide; tolcyclamide, glimepiride, nateglinide, or mitiglinide. In some embodiments the agent is a thiazolidinedione. In some embodiments the thiazolidinedione is rosiglitazone or pioglitazone. In some embodiments the thiazolidinedione is rosiglitazone. In some embodiments the metabolic-related disorder is dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance, obesity,

59.US1.PRO

## Provisional Application

impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, heart disease and type 2 diabetes. In some embodiments the metabolic-related disorder is dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance and type 2 diabetes. In some embodiments the metabolic-related disorder is dyslipidemia. In some  
5       embodiments the metabolic-related disorder is atherosclerosis. In some embodiments the metabolic-related disorder is coronary heart disease. In some embodiments the metabolic-related disorder is insulin resistance. In some embodiments the metabolic-related disorder is type 2 diabetes.

One aspect of the present invention encompasses a method of producing a  
10       pharmaceutical composition comprising admixing at least one compound according to Formula (I), as described herein, and a pharmaceutically acceptable carrier or excipient.

Applicant reserves the right to exclude any one or more of the compounds from any of the embodiments of the invention. Applicant additionally reserves the  
15       right to exclude any metabolic-related disorder from any of the embodiments of the invention.

These and other aspects of the invention disclosed herein will be set forth in greater detail as the patent disclosure proceeds.

## BRIEF DESCRIPTION OF THE DRAWINGS

20       **Figure 1.** Figure 1 depicts a histogram representing relative expression levels of **hRUP25** detected in different human tissues *via* DNA microarray. The horizontal axis displays the different tissues, identified in vertical text above the bar. The vertical axis indicates level of expression of **hRUP25**. In Figure 1, note the high level of expression  
25       in primary adipocytes of **hRUP25**.

**Figure 2.** Figure 2 depicts melanophores transfected with DNA plasmids expressing **hRUP25** without treatment. These cells are pigment-aggregated because **hRUP25** are Gi-coupled receptors having a high basal level of activity, and therefore driving the aggregation to a measurable level in the absence of a ligand.

30       **Figures 3A-B.** Figures 3A and 3B illustrate the dose-dependant, nicotinic acid induced aggregation response of melanophores transfected with increasing amounts of plasmid DNA encoding **hRUP25** (Figure 3A). Cells transfected with 10 $\mu$ g of plasmid DNA encoding **hRUP25**, respond to nicotinic acid with an EC<sub>50</sub> of about 54nM.

59.US1.PRO

## Provisional Application

As negative controls, Figure 3B depicts melanophores transfected with either salmon sperm DNA (Mock) or plasmid DNA encoding the  $\alpha_{2A}$ AR. As is evident there is no aggregation response in these cells upon nicotinic acid treatment at doses up to 10 $\mu$ M.

5       **Figure 4.** Figure 4 illustrates the nicotinic acid induced-inositol phosphates (IPs) accumulation in HEK293 cells co-expressing **hRUP25** and the chimeric G $\alpha$ q-subunit in which the last five amino acids have been replaced with the corresponding amino acids of G $\alpha$ i (Gq $\Delta$ Gi). This construct has been shown to convert the signaling of a Gi-coupled receptor to the Gq pathway (i.e. accumulation of inositol phosphates) in response to  
10       receptor activation. Cells transfected with Gq $\Delta$ Gi plus either empty plasmid or the constitutively activated  $\alpha_{2A}$ AR ( $\alpha_{2A}$ K) served as controls for the IP assay which are non-responsive to nicotinic acid.

15       **Figures 5A-B.** Figure 5A is a set of immunofluorescent photomicrographs illustrating the expression of hemagglutinin (HA)-tagged **hRUP25** in a stably transfected line of CHO cells (top; clone #46). No significant labeling is detected in mock stably-transfected CHO cells (Mock). The lower panels identify the nuclear (DAPI) staining of cells in the same field.

20       Figure 5B illustrates nicotinic acid and nicotine induced-inhibition of forskolin stimulated cAMP accumulation in **hRUP25**-CHO cell stable line #46 (described in preceding paragraph). The EC<sub>50</sub> for nicotinic acid is 23.6nM and that for nicotine is 9.8 $\mu$ M.

25       **Figure 6.** Figure 6 indicates that, in response to nicotinic acid, both **hRUP25** and the mouse ortholog **mRUP25** can inhibit TSHR stimulated cAMP production (in the presence and absence of TSH).

30       **Figure 7.** Figure 7 shows the saturation binding curves of [<sup>3</sup>H]nicotinic acid ([<sup>3</sup>H]NA) to membranes prepared from HEK293 cells transiently expressing either **hRUP25** or **mRUP25**. Note the significant binding of [<sup>3</sup>H]NA relative to either that found in membranes derived from mock transfected cells or in the presence of an excess of non-labeled nicotinic acid (200 $\mu$ M).

30       **Figure 8.** Figure 8 is a table comparing the rank order of potency of various compounds on **hRUP25** and the pharmacologically defined nicotinic acid receptor. The potencies at **hRUP25** derived both by a functional analysis measuring the inhibition of forskolin induced cAMP production and competitive radioligand binding

59.US1.PRO

## Provisional Application

assays, closely match the order of potencies of the pharmacologically defined nicotinic acid receptor.

Figures 9A-B. Figure 9A depicts nicotinic acid and related compounds inhibiting isoproterenol induced lipolysis in rat epididimal fat derived adipocytes at a concentration of 10 $\mu$ M. P-3-T represents 3-tetrazole-5-pyridine. Figure 9B illustrates a nicotinic acid dose-dependent inhibition of isoproterenol induced-lipolysis in rat epididimal fat derived adipocytes. Note the rightward shift in the dose-response curves with increasing concentrations of nicotinic acid.

Figure 10. Figure 10 illustrates the ability of both nicotinic acid and the related compound P-3-T (3-tetrazole-5-pyridine) to inhibit isoproterenol induced lipolysis in adipocyte primary cultures derived from human subcutaneous fat in a dose-dependant manner. The EC<sub>50</sub> value for nicotinic acid and P-3-T were 716nM and 218nM respectively.

## DETAILED DESCRIPTION OF THE INVENTION

## A. Definitions

The scientific literature that has evolved around receptors has adopted a number of terms to refer to ligands having various effects on receptors. For clarity and consistency, the following definitions will be used throughout this patent document. To the extent that these definitions conflict with other definitions for these terms, the following definitions shall control:

**AFFINITY REAGENTS** shall mean compounds that specifically and measurably bind to a target molecule. Preferably the target molecule is a GPCR of the invention. Preferably the **AFFINITY REAGENTS** are labeled to facilitate detection.

**AGONISTS** shall mean materials (e.g., ligands, candidate compounds) that activate an intracellular response when they bind to the receptor. In some embodiments, **AGONISTS** are those materials not previously known to activate the intracellular response when they bind to the receptor (e.g. to enhance GTP $\gamma$ S binding to membranes or to lower intracellular cAMP level). In some embodiments, **AGONISTS** are those materials not previously known to inhibit lipolysis when they bind to the receptor.

**ALLOSTERIC MODULATORS** shall mean materials (e.g., ligands, candidate compounds) that affect the functional activity of the receptor but which do not inhibit the

## 59.US1.PRO

## Provisional Application

endogenous ligand from binding to the receptor. Allosteric modulators include inverse agonists, partial agonists and agonists.

**ANTAGONISTS** shall mean materials (e.g., ligands, candidate compounds) that competitively bind to the receptor at the same site as the agonists but which do not activate an intracellular response, and can thereby inhibit the intracellular responses elicited by agonists. **ANTAGONISTS** do not diminish the baseline intracellular response in the absence of an agonist. In some embodiments, **ANTAGONISTS** are those materials not previously known to compete with an agonist to inhibit the cellular response when they bind to the receptor, e.g. wherein the cellular response is GTP $\gamma$ S binding to membranes or to the lowering of intracellular cAMP level.

**ANTILIPOLYTIC GPCR** shall mean a GPCR expressed by adipocytes and coupled to Gi or a Gi-coupled GPCR belonging to the nicotinic acid receptor sub-family of GPCRs. Activation of a Gi-coupled GPCR on adipocytes lowers intracellular cAMP levels, resulting in an inhibition of hormone sensitive lipase activity.

**ATHEROSCLEROSIS** is intended herein to encompass disorders of large and medium-sized arteries that result in the progressive accumulation within the intima of smooth muscle cells and lipids.

**CHEMICAL GROUP, MOIETY or RESIDUE** shall have the following meaning in the specification and Formulae described herein:

The term "**C<sub>1-5</sub> alkylene**" refers to a divalent branched or straight carbon group consisting of 1 to 5 carbon atoms, such as, -CH<sub>2</sub>-, -CH<sub>2</sub>CH<sub>2</sub>-, -CH(CH<sub>3</sub>)-, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-, -CH<sub>2</sub>CH(CH<sub>3</sub>)-, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-, and the like. In some embodiments the "**C<sub>1-5</sub> alkylene**" group contains one double bond, one triple bond or a carbonyl group whereby the one single bond of the "**C<sub>1-5</sub> alkylene**" group is replaced by a double bond or triple bond, such as, -CH=CH-, -C(CH<sub>3</sub>)=CH-, -CH=C(CH<sub>3</sub>)-, -CH<sub>2</sub>CH=CH-, -CH<sub>2</sub>CH<sub>2</sub>CH=CH-, -CH=CHCH<sub>2</sub>CH<sub>3</sub>-, and the like, when a double bond is present it can be *cis*, *trans* or a mixture of both; or one carbon of the "**C<sub>1-5</sub> alkylene**" group is the carbon of a carbonyl group, such as, -C(O)-, -CH<sub>2</sub>C(O)-, -C(O)CH<sub>2</sub>-, -C(O)CH(CH<sub>3</sub>)-, and the like.

The term "**C<sub>2-4</sub> alkenyl**" denotes a radical containing 2 to 4 carbons and at least one double bond. Some embodiments have 2 carbons. Examples of an alkenyl include vinyl, allyl, 2-butenyl, 3-butenyl and the like. Furthermore, the term "**alkenyl**" includes pure *cis* and *trans* isomers as well as mixtures thereof.

The term "**C<sub>1-4</sub> alkoxy**" as used herein denotes a radical alkyl, defined above, attached directly to an oxygen atom such as methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *t*-butoxy, *iso*-butoxy and the like.

5 The term "**C<sub>1-4</sub> alkyl**" denotes a radical containing 1 to 4 carbons unless otherwise specified. Some embodiments are 1 to 3 carbons, some embodiments are 1 to 2 carbons, and some embodiments have 1 carbon. Examples of an alkyl include methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *t*-butyl and the like.

10 The term "**C<sub>2-4</sub> alkynyl**" denotes a radical containing 2 to 4 carbons and at least one triple bond. Some embodiments have 2 carbons. Examples of an alkynyl include ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl, 3-butylnyl and the like.

15 The term "**C<sub>1-4</sub> alkylsulfinyl**" denotes a sulfoxide, i.e., -S(O)-, radical containing 1 to 4 carbons, linear or branched. Examples include methylsulfinyl, ethylsulfinyl and the like.

The term "**C<sub>1-4</sub> alkylsulfonyl**" denotes a sulfone, i.e., -S(O)<sub>2</sub>-, radical containing 1 to 4 carbons, linear or branched. Examples include methylsulfonyl, ethylsulfonyl and the like.

20 The term "**C<sub>1-4</sub> alkylthio**" denotes a sulfide, i.e., -S-, radical containing 1 to 4 carbons, linear or branched. Examples include methylsulfide, ethylsulfide, isopropylsulfide and the like.

The term "**amino**" denotes the group -NH<sub>2</sub>.

25 The term "**C<sub>1-4</sub> aminoalkyl**" denotes an amino substituted with one group selected from alkyl containing 1 to 4 carbon atoms. Some examples include methylamino, ethylamino, and the like.

The term "**C<sub>2-4</sub> aminodialkyl**" denotes an amino substituted with two alkyl radicals that can be same or different wherein the alkyls group can contain 1 to 4 carbon atoms. Some examples include dimethylamino, methylethylamino, diethylamino and the like.

30 The term "**cyano**" denotes the group -CN.

The term "**halo**" or "**halogen**" denotes to a fluoro, chloro, bromo or iodo group.

59.US1.PRO

## Provisional Application

The term "**C<sub>1-4</sub> haloalkoxy**" denotes a haloalkyl, as defined above, that is directly attached to an oxygen to form a difluoromethoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, pentafluoroethoxy and the like.

5 The term "**C<sub>1-4</sub> haloalkyl**" denotes an alkyl group as defined above that is substituted with one or more halogens, preferably fluorine, such as a fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, pentafluoroethyl and the like.

10 The term "**C<sub>1-4</sub> haloalkylsulfinyl**" denotes a sulfoxide, i.e., -S(O)-, radical containing 1 to 4 carbons substituted with one or more halogens, linear or branched. Examples include trifluoromethylsulfinyl, 2,2,2-trifluoroethylsulfinyl, 2,2-difluoroethylsulfinyl and the like.

15 The term "**C<sub>1-4</sub> haloalkylsulfonyl**" denotes a sulfone, i.e., -S(O)<sub>2</sub>-, radical containing 1 to 4 carbons, linear or branched substituted with one or more halogens. Examples include trifluoromethylsulfonyl, 2,2,2-trifluoroethylsulfonyl, 2,2-difluoroethylsulfonyl and the like.

The term "**C<sub>1-4</sub> haloalkylthio**" denotes an alkylthio radical substituted with one or more halogens. Examples include trifluoromethylthio, 1,1-difluoroethylthio, 2,2,2-trifluoroethylthio and the like.

20 The term "**heteroaryl**" denotes 5 or 6-membered aromatic rings having at least 1, 2, 3 or 4 heteroatoms in the ring, examples include, but are limited to, 1,3,4-oxadiazole, 1,2,4-oxadiazole, triazole, pyrazole, pyrrole, isoxazole, furane, thiophene, thiazole, oxazole, pyridyl, pyrazinyl, pyridazinyl, pyrimidinyl, triazinyl, and the like; in some embodiments the "**heteroaryl**" is further substituted with substituents as described herein.

25 The term "**hydroxyl**" denotes the group -OH.

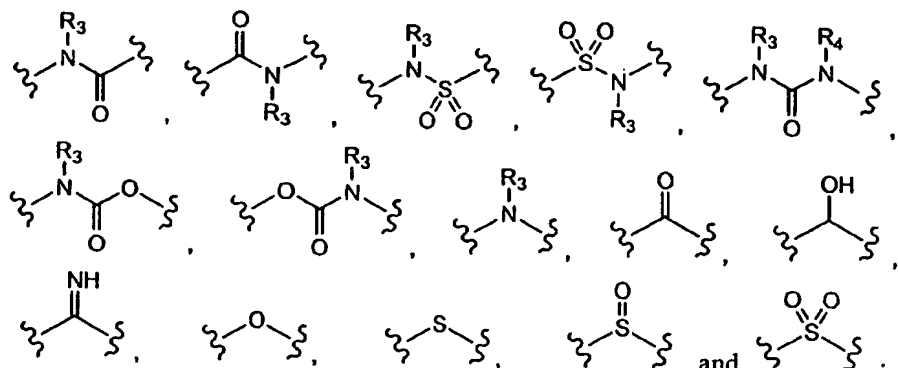
The term "**nitro**" denotes the group -NO<sub>2</sub>.

The term "**phenyl**" denotes the C<sub>6</sub>H<sub>5</sub>- group, in some embodiments the "**phenyl**" is further substituted with substituents as described herein.

The term "**thiol**" denotes the group -SH.

30 The variable "**X**" in Formulae found in this disclosure is selected from the group consisting of -NR<sub>3</sub>C(O)-, -C(O)NR<sub>3</sub>-, -NR<sub>3</sub>S(O)<sub>2</sub>-, -S(O)<sub>2</sub>NR<sub>3</sub>-, -NR<sub>3</sub>C(O)NR<sub>4</sub>-, -NR<sub>3</sub>C(O)O-, -OC(O)NR<sub>3</sub>-, -NR<sub>3</sub>-, -C(O)-, -CH(OH)-, -C(NH)-, -O-, -S-, -S(O)- or -S(O)<sub>2</sub>- and are represented respectively by the following:

## Provisional Application



5

10

15

20

25

59.US1.PRO

**Provisional Application**

**DECREASE** is used to refer to a reduction in a measurable quantity and is used synonymously with the terms "reduce", "diminish", "lower", and "lessen".

**DIABETES** as used herein is intended to encompass the usual diagnosis of **DIABETES** made from any of the methods including, but not limited to, the following list: symptoms of diabetes (e.g., polyuria, polydipsia, polyphagia) plus casual plasma glucose levels of greater than or equal to 200 mg/dl, wherein casual plasma glucose is defined any time of the day regardless of the timing of meal or drink consumption; 8 hour fasting plasma glucose levels of less than or equal to 126 mg/dl; and plasma glucose levels of greater than or equal to 200 mg/dl 2 hours following oral administration of 75 g anhydrous glucose dissolved in water.

**DIRECTLY IDENTIFYING** or **DIRECTLY IDENTIFIED**, in relationship to the phrase "candidate compound", shall mean the screening of a candidate compound against a constitutively activated receptor, preferably a constitutively activated orphan receptor, and most preferably against a constitutively activated G protein-coupled cell surface orphan receptor, and assessing the compound efficacy of such compound. This phrase is, under no circumstances, to be interpreted or understood to be encompassed by or to encompass the phrase "indirectly identifying" or "indirectly identified."

**DISORDERS OF LIPID METABOLISM** are intended herein to include, but not be limited to, dyslipidemia.

**DYSLIPIDEMIA** is intended herein to encompass disorders comprising any one of elevated level of plasma free fatty acids, elevated level of plasma cholesterol, elevated level of LDL-cholesterol, reduced level of HDL-cholesterol, and elevated level of plasma triglycerides.

**IN NEED OF PROPHYLAXIS OR TREATMENT** as used herein refers to a judgment made by a caregiver (e.g. physician, nurse, nurse practitioner, etc. in the case of humans; veterinarian in the case of animals, including non-human mammals) that an individual or animal requires or will benefit from prophylaxis or treatment. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that includes the knowledge that the individual or animal is ill, or will be ill, as the result of a disease, condition or disorder that is treatable by the compounds of the invention. In general, "in need of prophylaxis" refers to the judgment made by the caregiver that the individual will become ill. In this context, the compounds of the invention are used in a protective or preventive manner. However, "in need of treatment" refers to the judgment of the caregiver that the individual is already ill,

## 59.US1.PRO

## Provisional Application

therefore, the compounds of the present invention are used to alleviate, inhibit or ameliorate the disease, condition or disorder.

5 **INDIVIDUAL** as used herein refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans.

**INHIBIT** or **INHIBITING**, in relationship to the term "response" shall mean that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

10 **INSULIN RESISTANCE** as used herein is intended to encompass the usual diagnosis of insulin resistance made by any of a number of methods, including but not restricted to: the intravenous glucose tolerance test or measurement of the fasting insulin level. It is well known that there is an excellent correlation between the height of the fasting insulin level and the degree of insulin resistance. Therefore, one could use elevated fasting insulin levels as a surrogate marker for insulin resistance for the purpose of identifying which normal glucose tolerance (NGT) individuals have insulin resistance. A diagnosis of insulin resistance can also be made using the euglycemic glucose clamp test.

20 **INVERSE AGONISTS** shall mean materials (e.g., ligand, candidate compound) that bind either to the endogenous form or to the constitutively activated form of the receptor so as to reduce the baseline intracellular response of the receptor observed in the absence of agonists.

**KNOWN RECEPTOR** shall mean an endogenous receptor for which the endogenous ligand specific for that receptor has been identified.

25 **LIGAND** shall mean a molecule specific for a naturally occurring receptor.

**METABOLIC-RELATED DISORDERS** are intended herein to include, but not be limited to, dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance, obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, heart disease and type 2 diabetes.

30 As used herein, the terms **MODULATE** or **MODIFY** are meant to refer to an increase or decrease in the amount, quality, or effect of a particular activity, function or molecule.

As used herein, the term **NICOTINIC ACID ANALOG OR DERIVATIVE** is meant to molecules which bind to nicotinic acid receptors and have substantially similar

## 59.US1.PRO

## Provisional Application

effects on the receptor. Such analogs and derivatives are well-known to those skilled in the art and include, but are not limited to, acipimox and niacinamide.

5 **PARTIAL AGONISTS** shall mean materials (*e.g.*, ligands, candidate compounds) that activate the intracellular response when they bind to the receptor to a lesser degree/extent than do full agonists.

10 **PHARMACEUTICAL COMPOSITION** shall mean a composition comprising at least one active ingredient, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, and not limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

**STIMULATE** or **STIMULATING**, in relationship to the term "response" shall mean that a response is increased in the presence of a compound as opposed to in the absence of the compound.

15 **SUBJECT** shall mean primates, including but not limited to humans and baboons, as well as pet animals such as dogs and cats, laboratory animals such as rats and mice, and farm animals such as horses, sheep, and cows.

20 **SUBSTANTIALLY** shall refer to a result which is within 40% of a control result, preferably within 35%, more preferably within 30%, more preferably within 25%, more preferably within 20%, more preferably within 15%, more preferably within 10%, more preferably within 5%, more preferably within 2%, and most preferably within 1% of a control result. For example, in the context of receptor functionality, a test receptor may exhibit substantially similar results to a control receptor if the transduced signal, measured using a method taught herein or similar method known to the art-skilled, is

25 within 40% of the signal produced by a control signal.

The order of the following sections is set forth for presentational efficiency and is not intended, nor should be construed, as a limitation on the disclosure or the claims to follow.

30 **THERAPEUTICALLY EFFECTIVE AMOUNT** as used herein refers to the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal, individual or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes one or more of the following:

59.US1.PRO

## Provisional Application

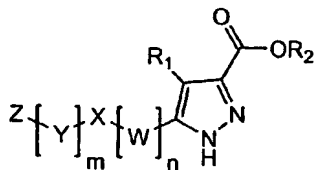
(1) Preventing the disease; for example, preventing a disease, condition or disorder in an individual that can be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease,

(2) Inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual that is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology), and

(3) Ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual that is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology).

## B. COMPOUNDS OF THE INVENTION

One aspect of the present invention encompasses pyrazole carboxylic acid and ester derivatives as shown in Formula (I):



(I)

wherein:

W and Y are independently a straight or branched chain  $C_{1-5}$  alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said  $C_{1-5}$  alkylene group is optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  haloalkyl or  $C_{1-4}$  alkoxy;

X is  $-NR_3C(O)-$ ,  $-C(O)NR_3-$ ,  $-NR_3S(O)_2-$ ,  $-S(O)_2NR_3-$ ,  $-NR_3C(O)NR_4-$ ,  $-NR_3C(O)O-$ ,  $-OC(O)NR_3-$ ,  $-NR_3-$ ,  $-C(O)-$ ,  $-CH(OH)-$ ,  $-C(NH)-$ ,  $-O-$ ,  $-S-$ ,  $-S(O)-$  or  $-S(O)_2-$ ;

$R_3$  and  $R_4$  are independently H,  $C_{1-4}$  alkyl, phenyl or heteroaryl, wherein each of said alkyl, phenyl or heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxyl, thiol, cyano, nitro,  $C_{1-4}$  haloalkyl, amino,  $C_{1-4}$  aminoalkyl,  $C_{2-4}$  aminodialkyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  alkoxy,  $C_{2-4}$

59.US1.PRO

## Provisional Application

alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

Z is H, halogen, phenyl or heteroaryl, wherein said phenyl and heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxy, thiol, cyano, nitro, C<sub>1-4</sub> haloalkyl, amino, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

R<sub>1</sub> is H, hydroxyl, halogen, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> haloalkyl;

R<sub>2</sub> is H or C<sub>1-3</sub> alkyl and

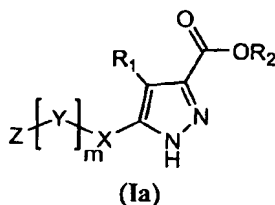
"n" and "m" or independently 0 or 1; or

a pharmaceutically acceptable salt, solvate or hydrate thereof;

provided that when X is -NR<sub>3</sub>- then "n" is not 0.

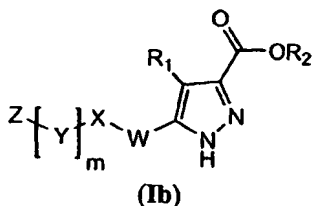
One embodiment of the present invention pertains to compounds of Formula (I)

wherein "n" is 0. This embodiment can be represented by Formula (Ia) as illustrated below:



wherein each variable in Formula (Ia) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein "n" is 1. This embodiment can be represented by Formula (Ib) as illustrated below:

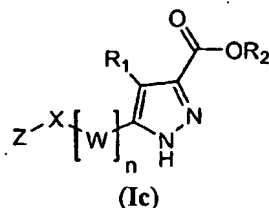


wherein each variable in Formula (Ib) has the same meaning as described herein, *supra* and *infra*.

59.US1.PRO

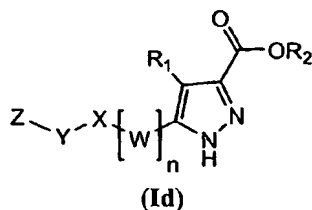
Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein "m" is 0. This embodiment can be represented by Formula (Ic) as illustrated below:



5 wherein each variable in Formula (Ic) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein "m" is 1. This embodiment can be represented by Formula (Id) as illustrated below:



10 wherein each variable in Formula (Id) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I), more specifically Formulae (Ib), (Ic) or (Id), wherein W is the straight or branched chain C<sub>1-5</sub> alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said C<sub>1-5</sub> alkylene group is optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. The C<sub>1-5</sub> alkylene group is a straight chain group of 1 to 5 carbons. In some embodiments the alkylene chain consists of single bonds. In some embodiments two adjacent carbons in this chain can be bonded together by a double bond or a triple bond, in still other embodiments, a single carbon can be bonded to an oxygen by a double bond thus forming a carbonyl group as represented in some embodiments disclosed herein [i.e., -C(=O)-].

20 The C<sub>1-5</sub> alkylene group, when present (i.e., n = 1), can be optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. The number of substituents on the C<sub>1-5</sub>

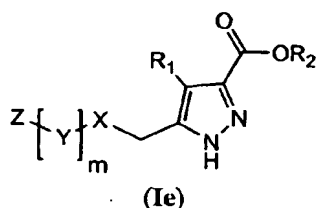
25

59.US1.PRO

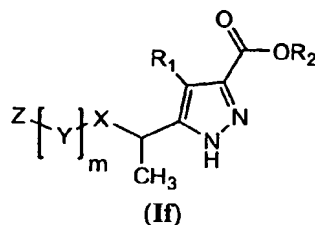
## Provisional Application

alkylene group depends on the specific group present, for example, when the C<sub>1-5</sub> alkylene group is -CH<sub>2</sub>- the number of substituents can be 1 or 2. Whereas, when the C<sub>1-5</sub> alkylene group is -(CH<sub>2</sub>)<sub>4</sub>- then the number of substituents can range from 1 to 8.

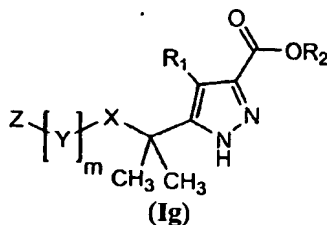
One embodiment of the present invention pertains to compounds of Formula (I) wherein W is -CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In one embodiment W is -CH<sub>2</sub>- and can be represented by Formula (Ie) as illustrated below:



wherein each variable in Formula (Ie) has the same meaning as described herein, *supra* and *infra*. In some embodiments W is -CH(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl or C<sub>1-4</sub> alkoxy. In one embodiment W is -CH(CH<sub>3</sub>)- and can be represented by Formula (If) as illustrated below:



wherein each variable in Formula (If) has the same meaning as described herein, *supra* and *infra*. In some embodiments W is -C(CH<sub>3</sub>)<sub>2</sub>-. This embodiment can be represented by Formula (Ig) as illustrated below:



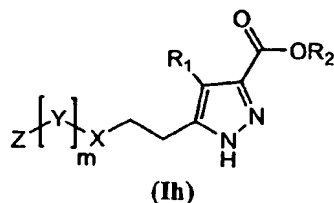
wherein each variable in Formula (Ig) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is -CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub>

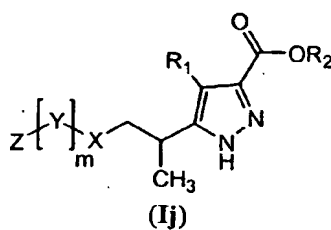
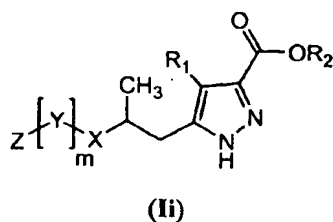
59.US1.PRO

## Provisional Application

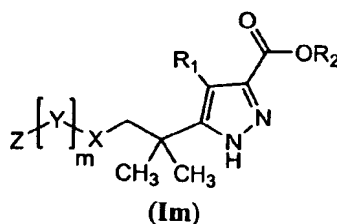
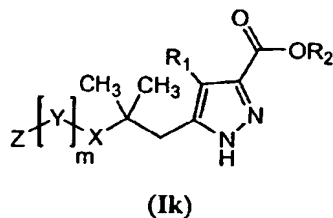
alkoxy. In one embodiment W is  $-\text{CH}_2\text{CH}_2-$  and can be represented by Formula (Ih) as illustrated below:



wherein each variable in Formula (Ih) has the same meaning as described herein, *supra* and *infra*. In some embodiments W is  $-\text{CH}(\text{CH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CH}(\text{CH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{CH}_3)-$  and can be represented by Formulae (Ii) and (Ij) respectively as illustrated below:



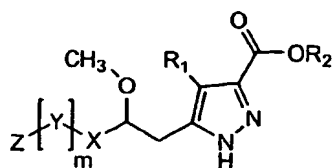
wherein each variable in Formulae (Ii) and (Ij) has the same meaning as described herein, *supra* and *infra*. In some embodiments W is  $-\text{C}(\text{CH}_3)_2\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{CH}_3)_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{C}(\text{CH}_3)_2\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{CH}_3)_2-$  and can be represented by Formulae (Ik) and (Im) respectively as illustrated below:



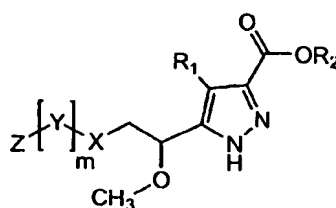
wherein each variable in Formulae (Ik) and (Im) has the same meaning as described herein, *supra* and *infra*. In some embodiments W is  $-\text{CH}(\text{OCH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{OCH}_3)-$  optionally substituted with halogen, hydroxyl or  $\text{C}_{1-4}$  alkyl. In some embodiments W is  $-\text{CH}(\text{OCH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{OCH}_3)-$  and can be represented by Formulae (In) and (Io) respectively as illustrated below:

59.US1.PRO

Provisional Application



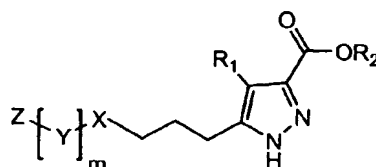
(In)



(Io)

wherein each variable in Formulae (In) and (Io) has the same meaning as described herein, *supra* and *infra*.

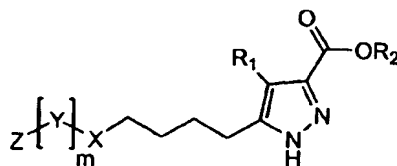
One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CHClCH}_2\text{CH}_2-$  or  $-\text{CH}_2\text{CH}_2\text{CHCl}-$ . In some embodiments W is  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  and can be represented by Formula (Ip) as illustrated below:



(Ip)

wherein each variable in Formula (Ip) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$  and can be represented by Formula (Iq) as illustrated below:



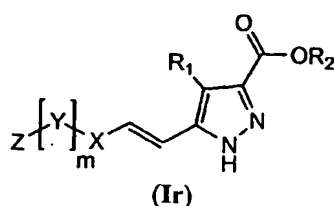
(Iq)

wherein each variable in Formula (Iq) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}=\text{CH}-$  optionally substituted with  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CH}=\text{CH}-$  and can be represented by Formula (Ir) as illustrated below:

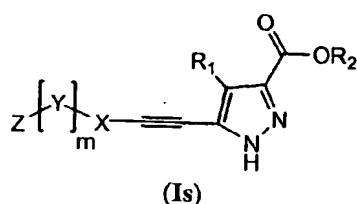
59.US1.PRO

## Provisional Application



wherein each variable in Formula (Ir) has the same meaning as described herein, *supra* and *infra*. It is understood that when a double bond is present it can be either *cis* or *trans*; or a mixture of both *cis* and *trans*.

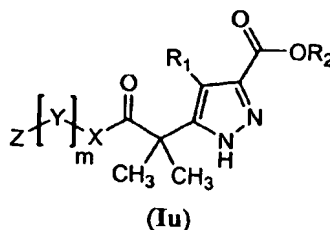
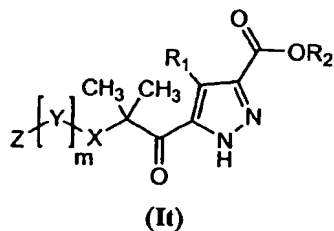
One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-C\equiv C-$  and can be represented by Formula (Is) as illustrated below:



wherein each variable in Formula (Is) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-C(O)-$ . In some embodiments, W is  $-C(O)-$  and X is  $-O-$ , in other embodiments, "m" is 1 thus forming an ester group and in still other embodiments "m" is 0 and Z is H to form a carboxylic acid group.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-CH_2C(O)-$  or  $-C(O)CH_2-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-CHFC(O)-$  or  $-C(O)CHF-$ . In some embodiments W is  $-CH(CH_3)C(O)-$  or  $-C(O)CH(CH_3)-$  optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. In some embodiments W is  $-C(OH)(CH_3)C(O)-$  or  $-C(O)C(OH)(CH_3)-$ . In some embodiments W is  $-C(CH_3)_2C(O)-$  or  $-C(O)C(CH_3)_2-$  and can be represented by Formulae (It) and (Iu) respectively as illustrated below:

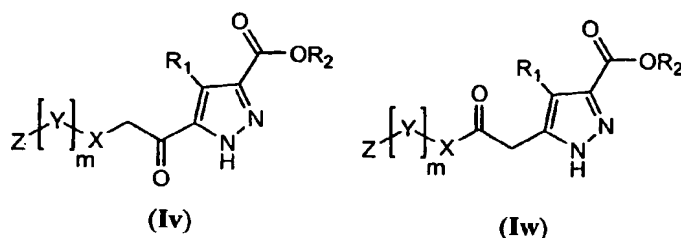


59.US1.PRO

## Provisional Application

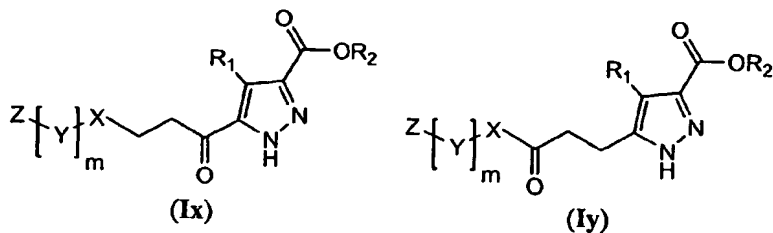
wherein each variable in Formulae (It) and (Iu) has the same meaning as described herein, *supra* and *infra*. Some embodiments of the present invention pertains to compounds of Formula (It) when X is -O- or -NR<sub>3</sub>-. Some embodiments of the present invention include compounds of Formula (Iu) when X is -O-. In some embodiments W is

5 -CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>- and can be represented by Formulae (Iv) and (Iw) respectively as illustrated below:



wherein each variable in Formulae (Iv) and (Iw) has the same meaning as described herein, *supra* and *infra*. Some embodiments of the present invention pertains to compounds of Formula (Iv) when X is -O- or -NR<sub>3</sub>-. Some embodiments of the present invention pertains to compounds of Formula (Iw) when X is -O-.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is -CH<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments W is -C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>C(CH<sub>3</sub>)<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments W is -CH<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>CH<sub>2</sub>- and can be represented by Formulae (Ix) and (Iy) respectively as illustrated below:



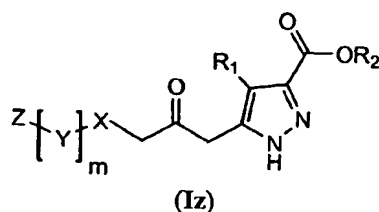
wherein each variable in Formulae (Ix) and (Iy) has the same meaning as described herein, *supra* and *infra*. Some embodiments of the present invention pertains to compounds of Formula (Ix) when X is -O- or -NR<sub>3</sub>- and in still other embodiments R<sub>3</sub> is H or CH<sub>3</sub>. Some embodiments of the present invention pertains to compounds of Formula (Iy) when X is

20 -O-.

## 59.US1.PRO

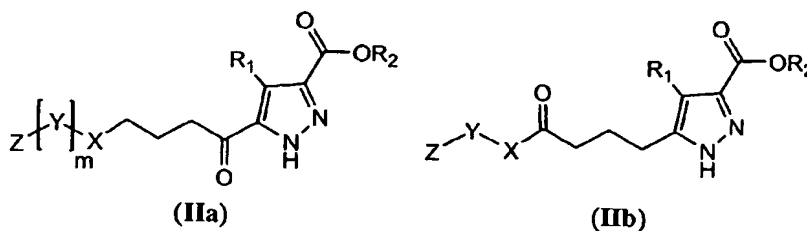
## Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  and can be represented by Formula (Iz) as illustrated below:



wherein each variable in Formulae (Iz) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_2-$  and can be represented by Formulae (IIa) and (IIb) respectively as illustrated below:

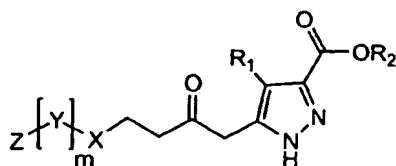


wherein each variable in Formulae (IIa) and (IIb) has the same meaning as described herein, *supra* and *infra*.

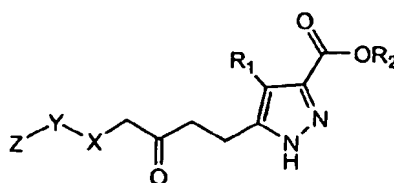
One embodiment of the present invention pertains to compounds of Formula (I) wherein W is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments W is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  and can be represented by Formulae (IIc) and (IId) respectively as illustrated below:

59.US1.PRO

## Provisional Application



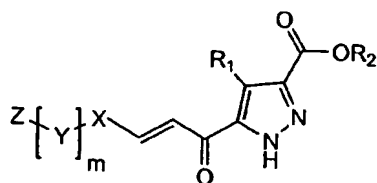
(IIc)



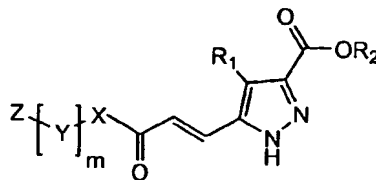
(IId)

wherein each variable in Formulae (IIc) and (IId) has the same meaning as described herein, *supra* and *infra*.

- One embodiment of the present invention pertains to compounds of Formula (I) wherein W is -CH=CHC(O)- or -C(O)CH=CH- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments W is -CH=CHC(O)- or -C(O)CH=CH- and can be represented by Formulae (IIe) and (IIIf) respectively as illustrated below:



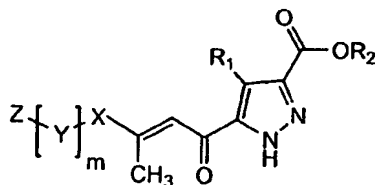
(IIe)



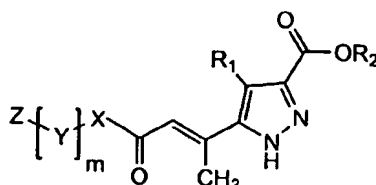
(IIIf)

- wherein each variable in Formulae (IIe) and (IIIf) has the same meaning as described herein, *supra* and *infra*.

- One embodiment of the present invention pertains to compounds of Formula (I) wherein W is -C(CH<sub>3</sub>)=CHC(O)- or -C(O)CH=C(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In one embodiment W is -C(CH<sub>3</sub>)=CHC(O)- or -C(O)CH=C(CH<sub>3</sub>)- and can be represented by Formulae (IIg) and (IIh) respectively as illustrated below:



(IIg)



(IIh)

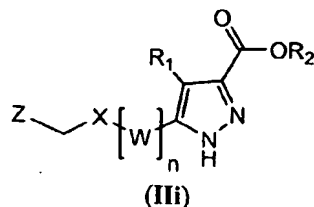
- wherein each variable in Formulae (IIg) and (IIh) has the same meaning as described herein, *supra* and *infra*.

## 59.US1.PRO

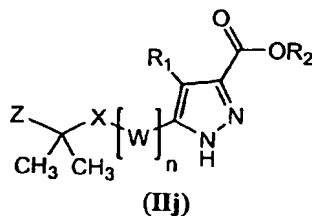
## Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is the C<sub>1-5</sub> alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said C<sub>1-5</sub> alkylene group is optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. The C<sub>1-5</sub> alkylene group is a straight chain group of 1 to 5 carbons. In some embodiments the alkylene chain consists of single bonds. In some embodiments two adjacent carbons in this chain can be bonded together by a double bond or a triple bond, in still other embodiments, a single carbon can be bonded to an oxygen by a double bond thus forming a carbonyl group as represented in some embodiments disclosed herein [i.e., -C(=O)-].

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is -CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments Y is -CH<sub>2</sub>- and can be represented by Formula (III) as illustrated below:



wherein each variable in Formula (III) has the same meaning as described herein, *supra* and *infra*. In some embodiments Y is -CH(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl or C<sub>1-4</sub> alkoxy. In some embodiments Y is -C(CH<sub>3</sub>)<sub>2</sub>- and can be represented by Formula (IIj) as illustrated below:

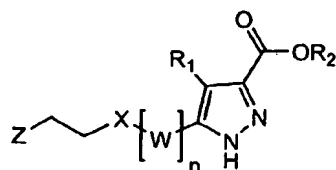


wherein each variable in Formula (IIj) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is -CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments Y is -CH<sub>2</sub>CH<sub>2</sub>- and can be represented by Formula (IIk) as illustrated below:

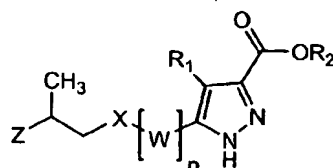
59.US1.PRO

## Provisional Application

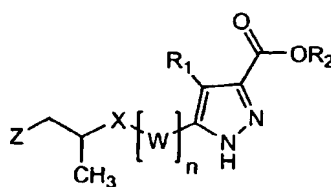


(IIk)

wherein each variable in Formula (IIk) has the same meaning as described herein, *supra* and *infra*. In some embodiments Y is -CH(CH<sub>3</sub>)CH<sub>2</sub>- or -CH<sub>2</sub>CH(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments Y is -CH(CH<sub>3</sub>)CH<sub>2</sub>- or -CH<sub>2</sub>CH(CH<sub>3</sub>)- and can be represented by Formulae (IIm) and (IIn) respectively as illustrated below:

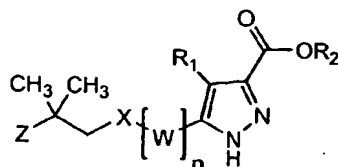


(IIm)

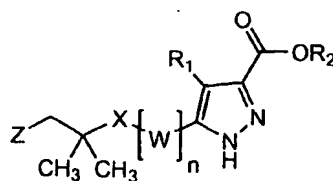


(IIn)

wherein each variable in Formulae (IIm) and (IIn) has the same meaning as described herein, *supra* and *infra*. In some embodiments Y is -C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>- or -CH<sub>2</sub>C(CH<sub>3</sub>)<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments Y is C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>- or -CH<sub>2</sub>C(CH<sub>3</sub>)<sub>2</sub>- and can be represented by Formulae (IIo) and (IIp) respectively as illustrated below:



(IIo)

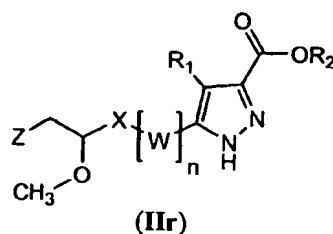
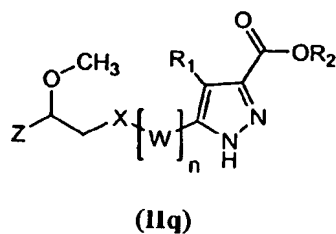


(IIp)

wherein each variable in Formulae (IIo) and (IIp) has the same meaning as described herein, *supra* and *infra*. In some embodiments Y is -CH(OCH<sub>3</sub>)CH<sub>2</sub>- or -CH<sub>2</sub>CH(OCH<sub>3</sub>)- optionally substituted with halogen, hydroxyl or C<sub>1-4</sub> alkyl. In some embodiments Y is -CH(OCH<sub>3</sub>)CH<sub>2</sub>- or -CH<sub>2</sub>CH(OCH<sub>3</sub>)- and can be represented by Formulae (IIq) and (IIr) respectively as illustrated below:

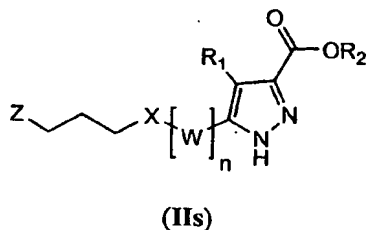
59.US1.PRO

## Provisional Application



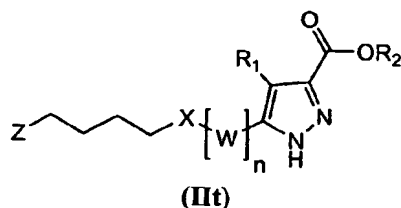
wherein each variable in Formulae (IIq) and (IIr) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  and can be represented by Formula (IIs) as illustrated below:



wherein each variable in Formula (IIs) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$  and can be represented by Formula (IIt) as illustrated below:

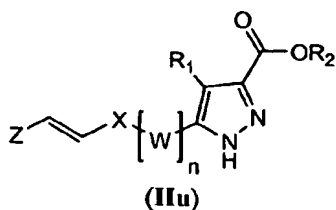


wherein each variable in Formula (IIt) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}=\text{CH}-$  optionally substituted with  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}=\text{CH}-$  and can be represented by Formula (IIu) as illustrated below:

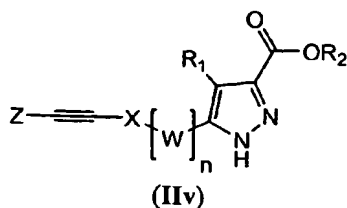
59.US1.PRO

Provisional Application



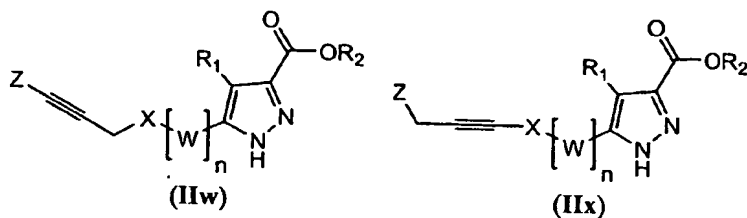
wherein each variable in Formula (IIu) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is -C≡C- and can be represented by Formula (IIv) as illustrated below:



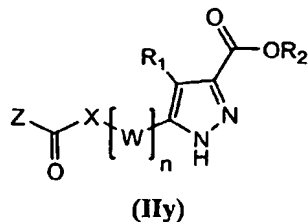
wherein each variable in Formula (IIv) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is -C≡CCH<sub>2</sub>- or -CH<sub>2</sub>C≡C- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments Y is -C≡CCH<sub>2</sub>- or -CH<sub>2</sub>C≡C- and can be represented by Formulae (IIw) and (IIx) respectively as illustrated below:



wherein each variable in Formulae (IIw) and (IIx) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is -C(O)- and can be represented by Formula (IIy) as illustrated below:

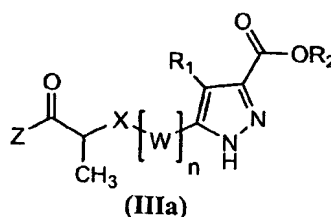
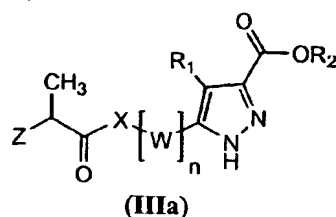


## 59.US1.PRO

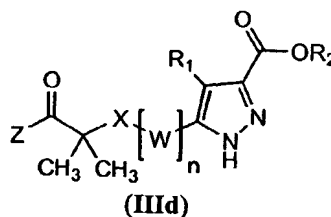
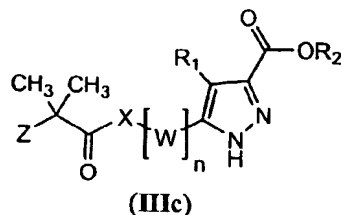
## Provisional Application

wherein each variable in Formula (IIy) has the same meaning as described herein, *supra* and *infra*.

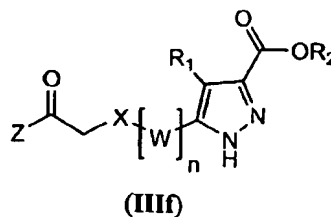
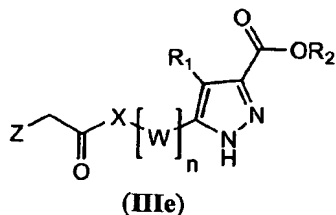
One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}(\text{CH}_3)\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}(\text{CH}_3)\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}(\text{CH}_3)-$  and can be represented by Formulae (IIIa) and (IIIb) respectively as illustrated below:



wherein each variable in Formulae (IIIa) and (IIIb) has the same meaning as described herein, *supra* and *infra*. In some embodiments Y is  $-\text{C}(\text{CH}_3)_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{C}(\text{CH}_3)_2-$  and can be represented by Formulae (IIIc) and (IIId) respectively as illustrated below:



wherein each variable in Formulae (IIIc) and (IIId) has the same meaning as described herein, *supra* and *infra*. In some embodiments Y is  $-\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2-$  and can be represented by Formulae (IIIe) and (IIIf) respectively as illustrated below:



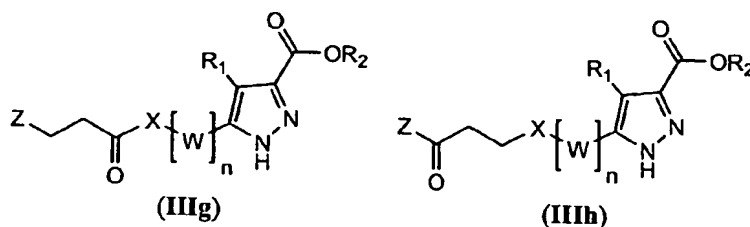
wherein each variable in Formulae (IIIe) and (IIIf) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{C}(\text{CH}_3)_2\text{CH}_2\text{C}(\text{O})-$  or

59.US1.PRO

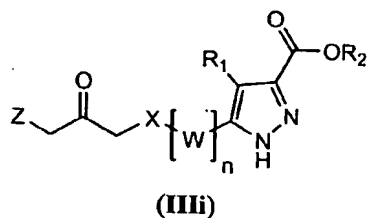
## Provisional Application

$-\text{C}(\text{O})\text{CH}_2\text{C}(\text{CH}_3)_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  and can be represented by Formulae (IIIg) and (IIIh) respectively as illustrated below:



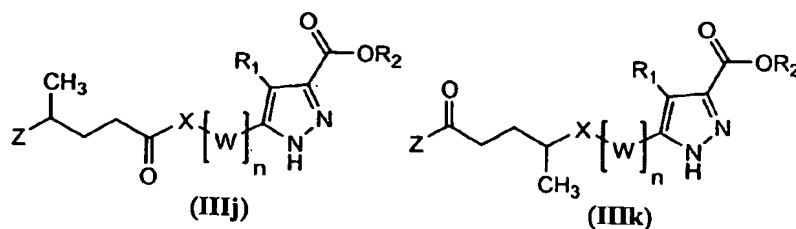
wherein each variable in Formulae (IIIg) and (IIIh) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  and can be represented by Formula (IIIi) as illustrated below:



wherein each variable in Formula (IIIi) has the same meaning as described herein, *supra* and *infra*.

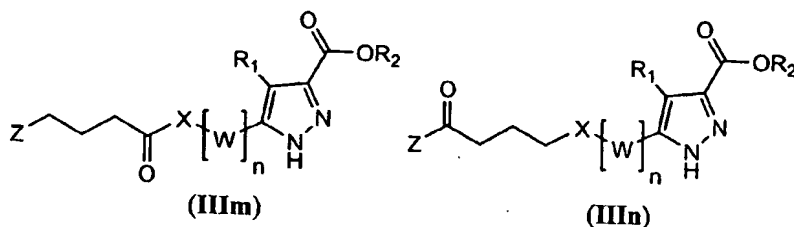
One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)-$  and can be represented by Formulae (IIIj) and (IIIk) respectively as illustrated below:



## 59.US1.PRO

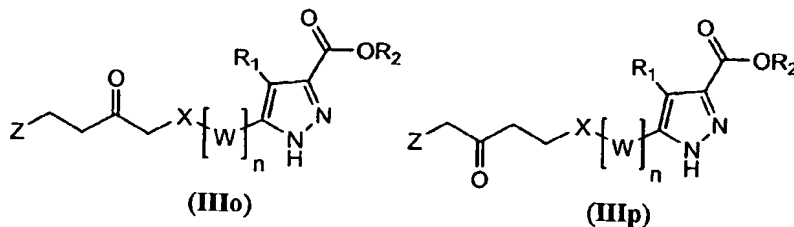
## Provisional Application

wherein each variable in Formulae (IIIj) and (IIIk) has the same meaning as described herein, *supra* and *infra*. In some embodiments Y is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_2-$  and can be represented by Formulae (IIIm) and (IIIn) respectively as illustrated below:



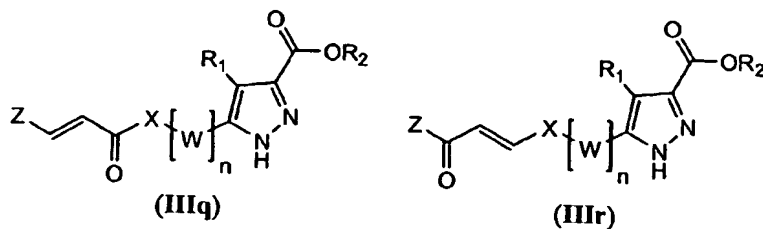
wherein each variable in Formulae (IIIm) and (IIIn) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{O})\text{CH}_2\text{CH}_2-$  and can be represented by Formulae (IIIo) and (IIIp) respectively as illustrated below:



wherein each variable in Formulae (IIIo) and (IIIp) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is  $-\text{CH}=\text{CHC}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}=\text{CH}-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy. In some embodiments Y is  $-\text{CH}=\text{CHC}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}=\text{CH}-$  and can be represented by Formulae (IIIq) and (IIIr) respectively as illustrated below:

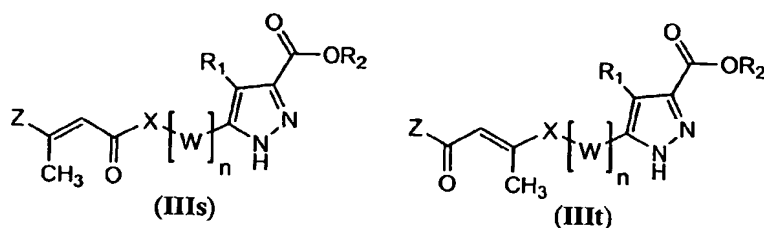


59.US1.PRO

## Provisional Application

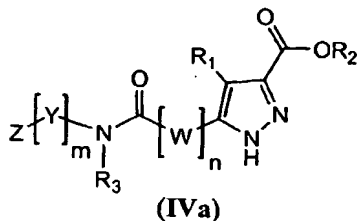
wherein each variable in Formulae (IIIq) and (IIIr) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Y is -C(CH<sub>3</sub>)=CHC(O)- or -C(O)CH=C(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. In some embodiments Y is -C(CH<sub>3</sub>)=CHC(O)- or -C(O)CH=C(CH<sub>3</sub>)- and can be represented by Formulae (IIIs) and (IIIt) respectively as illustrated below:



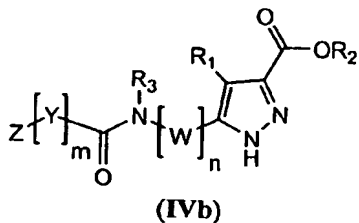
wherein each variable in Formulae (IIIs) and (IIIt) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -NR<sub>3</sub>C(O)- and can be represented by Formula (IVa) as illustrated below:



wherein each variable in Formula (IVa) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -C(O)NR<sub>3</sub>- and can be represented by Formula (IVb) as illustrated below:

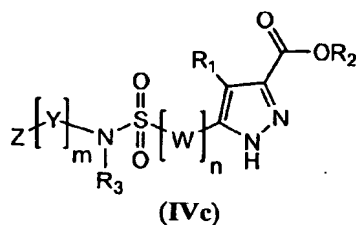


wherein each variable in Formula (IVb) has the same meaning as described herein, *supra* and *infra*.

## 59.US1.PRO

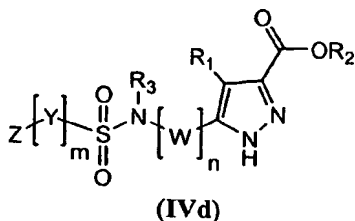
## Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3\text{S}(\text{O})_2-$  and can be represented by Formula (IVc) as illustrated below:



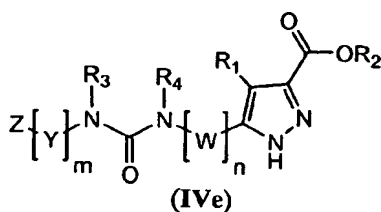
5 wherein each variable in Formula (IVc) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{S}(\text{O})_2\text{NR}_3-$  and can be represented by Formula (IVd) as illustrated below:



10 wherein each variable in Formula (IVd) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3\text{C}(\text{O})\text{NR}_4-$  and can be represented by Formula (IVe) as illustrated below:

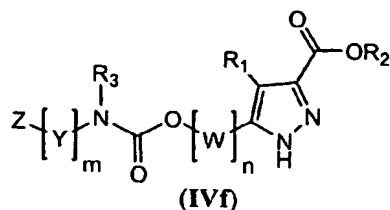


15 wherein each variable in Formula (IVe) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is  $-\text{NR}_3\text{C}(\text{O})\text{O}-$  and can be represented by Formula (IVf) as illustrated below:

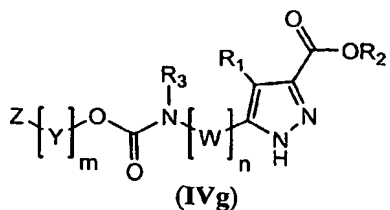
59.US1.PRO

Provisional Application



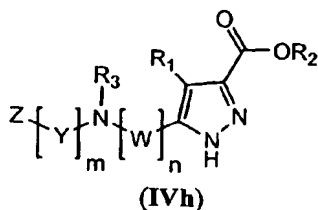
wherein each variable in Formula (IVf) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -OC(O)NR<sub>3</sub>- and can be represented by Formula (IVg) as illustrated below:



wherein each variable in Formula (IVg) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -NR<sub>3</sub>- and can be represented by Formula (IVh) as illustrated below:



wherein each variable in Formula (IVh) has the same meaning as described herein, *supra* and *infra*.

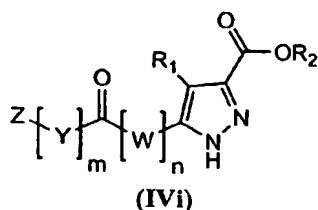
One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>3</sub> is H or CH<sub>3</sub>.

One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>4</sub> is H or CH<sub>3</sub>.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -C(O)- and can be represented by Formula (IVi) as illustrated below:

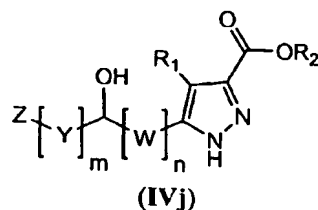
59.US1.PRO

## Provisional Application



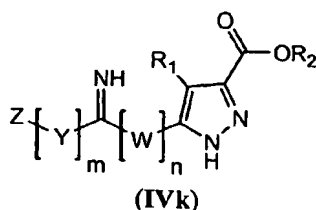
wherein each variable in Formula (IVi) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -CH(OH)- and can be represented by Formula (IVj) as illustrated below:



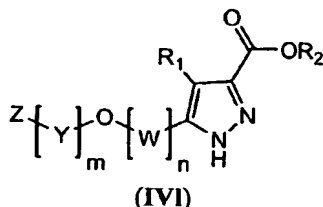
wherein each variable in Formula (IVj) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -C(NH)- and can be represented by Formula (IVk) as illustrated below:



wherein each variable in Formula (IVk) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -O- and can be represented by Formula (IVl) as illustrated below:

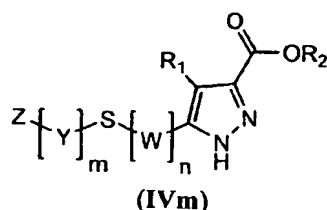


wherein each variable in Formula (IVl) has the same meaning as described herein, *supra* and *infra*.

59.US1.PRO

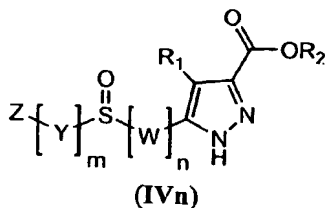
Provisional Application

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -S- and can be represented by Formula (IVm) as illustrated below:



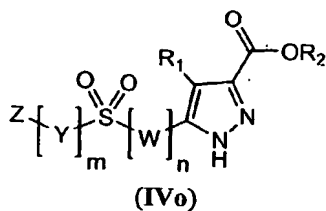
wherein each variable in Formula (IVm) has the same meaning as described herein,  
 5 *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -S(O)- and can be represented by Formula (IVn) as illustrated below:



wherein each variable in Formula (IVn) has the same meaning as described herein,  
 10 *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein X is -S(O)<sub>2</sub>- and can be represented by Formula (IVo) as illustrated below:



wherein each variable in Formula (IVo) has the same meaning as described herein,  
 15 *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is H.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is halogen.

20 One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is phenyl. In some embodiments the phenyl is optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub>

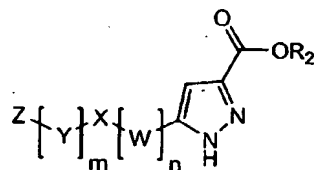
59.US1.PRO

Provisional Application

aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl. In some embodiments the phenyl is optionally substituted with 1 to 3 substituents selected from the group consisting of -F, -Cl, -Br, -CF<sub>3</sub>, -NHCH<sub>3</sub>, -N(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, -OCH<sub>3</sub> and -OCF<sub>3</sub>.

One embodiment of the present invention pertains to compounds of Formula (I) wherein Z is heteroaryl. In some embodiments the heteroaryl is optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl. In some embodiments the phenyl is optionally substituted with 1 to 3 substituents selected from the group consisting of -F, -Cl, -Br, -CF<sub>3</sub>, -NHCH<sub>3</sub>, -N(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, -OCH<sub>3</sub> and -OCF<sub>3</sub>.

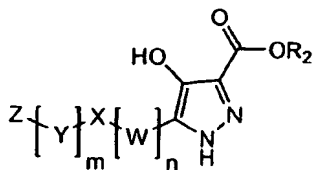
One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is H and can be represented by Formula (IVp) as illustrated below:



(IVp)

wherein each variable in Formula (IVp) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is hydroxyl and can be represented by Formula (IVq) as illustrated below:



(IVq)

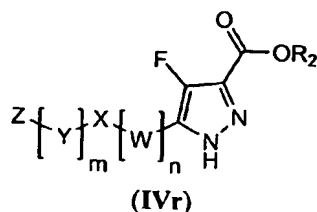
wherein each variable in Formula (IVq) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein R<sub>1</sub> is halogen. In some embodiments R<sub>1</sub> is F, Cl or Br. In still further

59.US1.PRO

## Provisional Application

embodiments,  $R_1$  is F (a fluorine atom) and can be represented by Formula (IVr) as illustrated below:

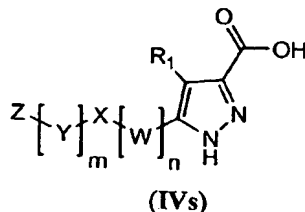


wherein each variable in Formula (IVr) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein  $R_1$  is  $C_{1-4}$  alkyl.

One embodiment of the present invention pertains to compounds of Formula (I) wherein  $R_1$  is  $C_{1-4}$  haloalkyl.

One embodiment of the present invention pertains to compounds of Formula (I) wherein  $R_2$  is H and can be represented by Formula (IVs) as illustrated below:



wherein each variable in Formula (IVs) has the same meaning as described herein, *supra* and *infra*.

One embodiment of the present invention pertains to compounds of Formula (I) wherein  $R_2$  is  $C_{1-8}$  alkyl.

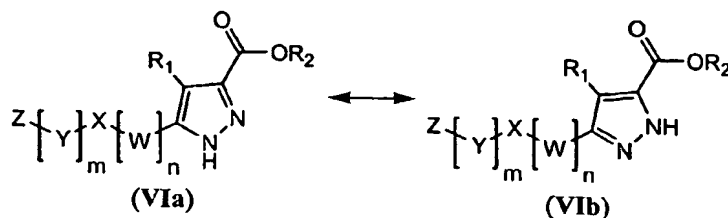
## CHEMISTRY OF THE PRESENT INVENTION

## Tautomers

Although compounds of the present invention of Formula (I) are depicted as one compound, it is well understood and appreciated in the art that pyrazoles can exist in various tautomeric forms. Two possible tautomeric forms are illustrated below:

59.US1.PRO

## Provisional Application



Accordingly, tautomeric forms can have corresponding nomenclature, for example, Formula (VIa) and Formula (VIb) can be represented by the general chemical names 1H-pyrazole and 2H-pyrazole respectively. Therefore, for convenience, compounds presented herein by Formula (I) are understood to include all tautomers and furthermore, these tautomers and various nomenclature designations are within the scope of the present invention.

**Enantiomers, Diastereomers and mixtures thereof:**

Compounds of Formula (I) may have one or more chiral centers, and therefore exist as enantiomers or diastereomers. The invention is understood to extend to all such enantiomers, diastereomers and mixtures thereof, including racemates. Formula (I) and the formulae described herein, *supra*, are intended to represent all individual isomers and mixtures thereof, unless stated or shown otherwise.

Racemic mixtures can be resolved into the optical pure enantiomers by known methods, for example, by separation of diastereomeric salts thereof with an optically active acid, and liberating the optically active amine compound by treatment with a base. Another method for resolving racemates into the optical pure enantiomers is based upon chromatography on an optically active matrix or chiral support. Certain racemic compounds of the present invention can thus be resolved into their optical antipodes, e.g., by fractional crystallization of d- or l- (tartrates, mandelates, or camphorsulphonate) salts for example. The compounds of the present invention may also be resolved by the formation of diastereomeric amides or ester by reaction of the compounds of the present invention with an optically active activated carboxylic acid such as that derived from (+) or (-) phenylalanine, (+) or (-) phenylglycine, (+) or (-) camphoric acid or by the formation of diastereomeric carbamates by reaction of the compounds of the present invention with an optically active chloroformate or the like subsequently hydrolyzed.

Additional methods for the resolution of optical isomers, known to those skilled in the art can be used, and will be apparent to the average worker skilled in the art. Such

59.US1.PRO

# Provisional Application

methods include those discussed by J. Jaques, A. Collet, and S. Wilen in "Enantiomers, Racemates, and Resolutions", John Wiley and Sons, New York (1981).

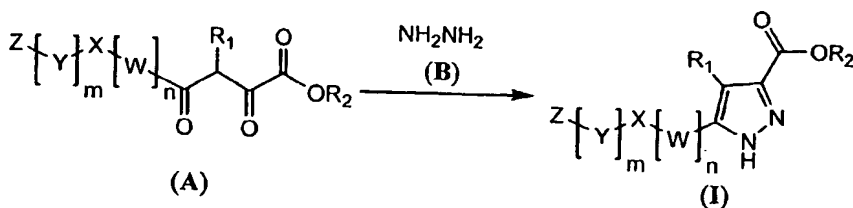
## Synthesis of Compounds of Formula (I)

The compounds of the present invention can be readily prepared according to a variety of synthetic regimes, all of which would be familiar to one skilled in the art. The chemical and patent literature quotes numerous procedures for the synthesis of pyrazole carboxylic acids and esters. Some of these articles include: Ashton and co-workers, *J. Med. Chem.* **1993**, *36*, 3595-3605; Seki and co-workers, *Chem. Pharm. Bull.*, **1984**, *32*, 1568; and Wiley and Hexner, *Org. Syn Coll IV*, **1963**, 351.

Also provided is a novel procedure for the preparation of novel pyrazoles of Formula (I).

In the illustrated syntheses outlined below, the labeled substituents have the same identifications as set out in the definitions of the compound described above for Formula (I). The methods described below can be used for the preparation of compounds of the invention.

One method that can be used to prepare compounds of Formula (I) utilizes intermediates of Formula (A) as illustrated in Reaction Scheme (1) below:



Reaction Scheme (1)

Compounds of Formula (I) can be prepared by treating the 2,4-diketo ester or acid [ $R_2$  = alkyl or H respectively, (A)] with hydrazine (B) under various conditions. For example, the solvent may optionally be present or absent. In the instance that the solvent is absent then the hydrazine (B) serves both as a reactant and as the solvent. Typically, under these conditions hydrazine would be present in molar excess. In the instance when the solvent is present, the solvent can be a polar solvent and is generally a  $C_1$ - $C_6$  alcohol. Some typical solvents can be selected from, but not limited to, the group consisting of methanol, ethanol, butanol, pentanol, hexanol, 2-methoxyethanol, 1-propanol and 2-propanol. In some instances it can be beneficial to include the presence of an acid. Some representative examples of acids that can be used can be selected from

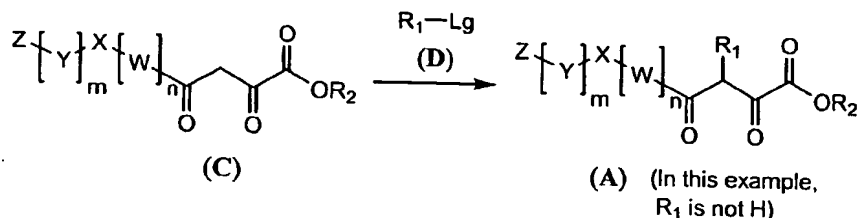
59.US1.PRO

Provisional Application

the group consisting of hydrochloric acid, hydrobromic acid, acetic acid and trifluoroacetic acid. The reaction temperature generally ranges from about 20°C to about 160°C, and for convenience, the reaction temperature is typically the reflux temperature of the reaction mixture.

5 The 2,4-diketo esters or acids (A) can be readily available or can be obtained by methods known in the art, Seki and co-workers, *Chem. Pharm. Bull.*, 1984, 32, 1568. It is appreciated that a group on the Z-[Y]<sub>m</sub>-X-[W]<sub>n</sub> chain of (A) can be protected by methods known in the art if such protection is required.

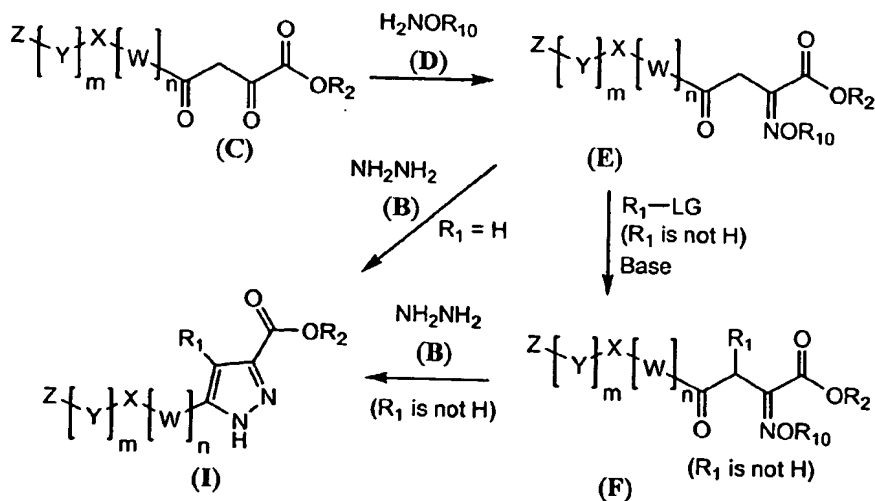
10 One particular feature of 2,4-diketo esters or acids (A) is that a diverse number of R<sub>1</sub> groups can be introduced by a variety of methods known in the art, such as, alkylation, as shown in Reaction Scheme (2) below:



Reaction Scheme (2)

The alkylation step as shown in Reaction Scheme (2) is similar to and in some instances identical to that described in the preparation of intermediate (F), *infra*.

15 Utilizing a similar starting material as in Reaction Scheme (1) an alternative method can be used to prepare compounds of Formula (I) as illustrated in Reaction Scheme (3) below:



Reaction Scheme (3)

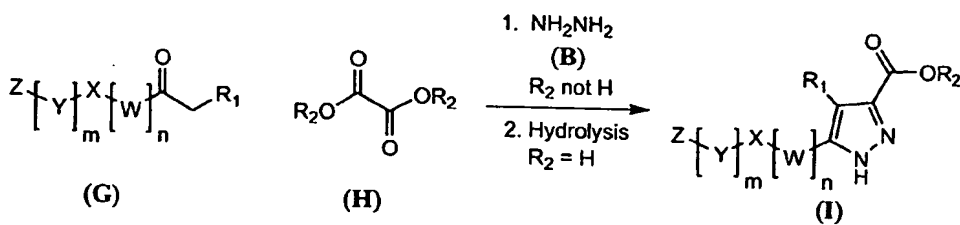
## 59.US1.PRO

## Provisional Application

Compounds of Formula (I) can be prepared by treating 2,4-diketo ester or acid (C), preferably the ester, with an alkoxyamine of Formula (D) where  $R_{10}$  is  $C_1$ - $C_8$  alkyl, leading to 2-(methoxyimino) intermediate of Formula (E). Typically, the alkoxyamine is methoxyamine (i.e., O-methyl hydroxylamine) wherein  $R_{10}$  is methyl. This step is typically conducted in the presence of a drying agent to concomitantly remove the water formed during the process; examples of a drying agent that can be used include molecular sieves, magnesium sulfate and the like. In the subsequent step, the intermediate of Formula (E) can be functionalized with  $R_1$  utilizing methods known in the art. One example may use  $R_1$ -LG, wherein LG is a leaving group, such as, iodo, bromo, mesylate and the like, in the presence of a base and a polar solvent. Typical bases can be selected from, potassium carbonate, sodium carbonate, sodium hydroxide, potassium hydroxide, lithium hydroxide, LDA, sodium methoxide, sodium ethoxide and the like; and the polar solvent can be dimethylformamide, dimethylsulfoxide, THF and the like. It is understood that this step is optional since in some embodiments of the invention  $R_1$  is H. In this context, as depicted in Reaction Scheme (3), intermediate (E) can be converted to a compound of Formula (I) wherein  $R_2$  is H using hydrazine (B). This step can be performed under heating conditions in an alcoholic solvent as described above in Reaction Scheme (1). Optionally, an acid can be present, such as HCl. Similarly, in the example where  $R_1$  is not H, intermediate (F) can be treated with hydrazine (B) in a manner as described above to provide compounds of Formula (I) where  $R_1$  is a group other than H.

It is understood that in reference to Reaction Scheme (3), a compound of Formula (C) can be functionalized with  $R_1$  as described above prior to treating with alkoxyamine (D) to give the same intermediate (F). Absent any chemical reason that would be known in the art, the order of the steps can be changed and can be more a matter of convenience than necessity [i.e., (C) to (E) to (F); or (C) to (A) to (F)].

A novel method for the preparation of compounds of Formula (I) is shown in Reaction Scheme (4).



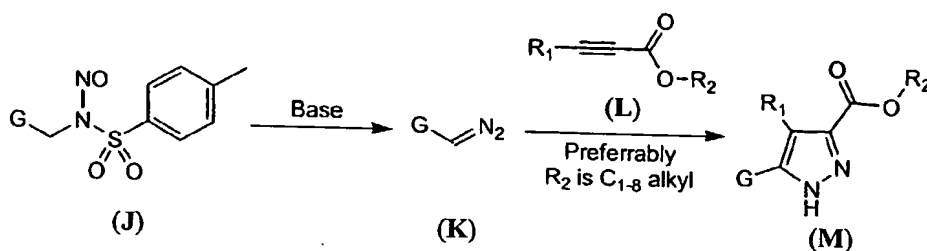
Reaction Scheme (4)

59.US1.PRO

## Provisional Application

In this procedure, ketone (G) is allowed to react with a base, such as sodium methoxide or other alkali metal alkoxide, in the presence of oxalate (H). The resulting mixture is treated with hydrazine (B) to compounds of the present invention wherein R<sub>2</sub> is alkyl. As an optional step, the ester can be converted to the carboxylic acid by methods known in the art.

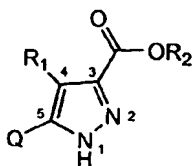
Another method for the preparation of compounds of Formula (I) is set forth in Reaction Scheme (5) and is intended to be illustrative and not limited.



Reaction Scheme (5)

Compounds of Formula (I) can be prepared by treating a nitroso sulfonamide (L) with base to give a substituted diazoalkane (K). A wide variety of functionality can be present in this synthesis, for example, G can be (CH<sub>3</sub>O)<sub>2</sub>CH- as shown in Example *infra*. Once formed, the substituted diazoalkane (K) can undergo a cycloaddition process with alkyne (L) to give pyrazole (M). A variety of alkynes can be either prepared or purchased from commercial sources to introduce the R<sub>1</sub> group as defined herein. Preferably, R<sub>1</sub> in Reaction Scheme (5) is H, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> haloalkyl.

It is understood that various pyrazoles can be prepared with groups present at the 5-position and these groups can be further converted or modified using methods known in the art into compounds of Formula (I).



For example, Q can be a group represented by formula H<sub>2</sub>N-[W]<sub>n</sub>, wherein "W" and "n" have the same meaning as used herein. The amine group can be modified with a variety of substituted aldehydes or ketones, such as those commercially available or prepared by methods known in the art, through a reductive amination procedure or similar method. Further, the amine can also be alkylated with Z-[Y]<sub>m</sub>-Lg wherein Lg is a leaving group as defined herein, *supra*. In addition, the amine can also be modified

59.US1.PRO

## Provisional Application

with a variety of electrophils, such as  $Z-[Y]_m-C(O)-Lg$  (i.e., an acid halide or anhydride),  $Z-[Y]_m-S(O)_2-Lg$  (i.e., a sulfonyl halide) or  $Z-[Y]_m-N=C=O$ . In the examples where  $R_3$  is not H, the amine can be further substituted with the  $R_3$  group via methods known in the art. In another example, Q can be  $HX-[W]_n-$  wherein X is "-O-" or "-S-" and can be modified via methods known in the art to give compounds of Formula (I), for example, via an alkylation procedure with  $Z-[Y]_m-Lg$  wherein Lg is a leaving group defined herein, *supra* or via the Mitsunobu reaction. In the scenario where X is "-S-", conversion can to the corresponding sulfoxide [i.e.,  $X = -S(O)-$ ] or sulfone [i.e.,  $X = -S(O)_2-$ ] can be implemented via oxidation, for example, mCPBA or  $H_2O_2$ . It is understood that the chemistry described for the Q group can be "reversed" or used in an "alternative" manner with the corresponding reactant. To illustrate this point, the aldehyde or ketone group can alternatively be part of the Q group and modified with a variety of amines using the similar synthetic procedures described above, such as, reductive amination (see Examples, *infra*). Likewise, in an alternative manner, Q can be  $Lg-[W]_n-$  and used, for example, to alkylate  $Z-[Y]_m-XH$ .

Esters of the compounds shown herein, wherein  $R_2 = \text{alkyl}$ , can be readily converted to the corresponding carboxylic acids of Formula (I) by methods known in the art, such as alkaline hydrolysis using LiOH, NaOH, KOH, and the like. Another method for the conversion of an ester to a carboxylic acid of Formula (I) is through the use of acid hydrolysis, such as aqueous HCl and the like. Generally, the solvent is an aqueous mixture with a polar solvent as described above.

The various organic group transformations and protecting groups utilized herein can be performed by a number of procedures other than those described above. References for other synthetic procedures that can be utilized for the preparation of intermediates or compounds disclosed herein can be found in, for example, Smith, M. B.; and March, J., *Advanced Organic Chemistry*, 5<sup>th</sup> Edition, Wiley-Interscience (2001); Larock, R.C., *Comprehensive Organic Transformations, A Guide to Functional Group Preparations*, 2<sup>nd</sup> Edition, VCH Publishers, Inc. (1999), or Wuts, P. G. M.; Greene, T. W.; *Protective Groups in Organic Synthesis*, 3<sup>rd</sup> Edition, John Wiley and Sons, (1999), all three incorporated in their entirety herein by reference.

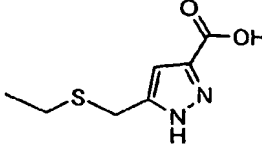
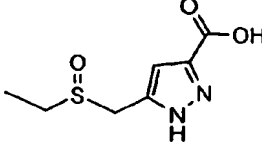
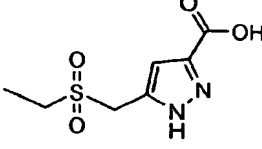
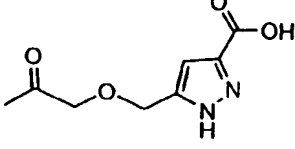
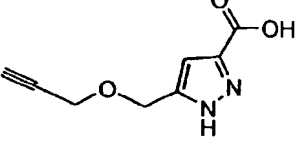
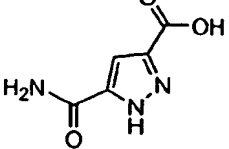
It is understood that the chemistry described here is representative and is not intended to be limiting in any manner.

Representative examples of compound of Formula (I) are shown below in TABLE A.

59.US1.PRO

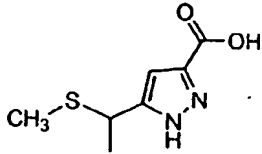
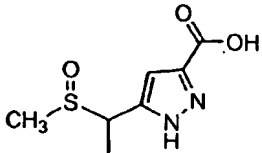
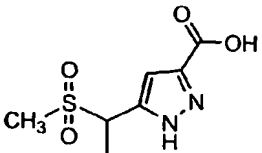
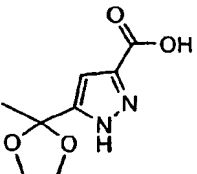
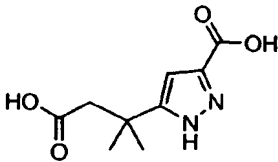
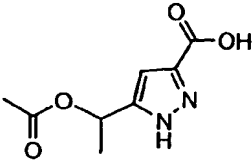
Provisional Application

TABLE A

Cmpd#	Structure	Chemical Name
1		5-Ethylsulfanylmethyl-1H-pyrazole-3-carboxylic acid
2		5-Ethanesulfinylmethyl-1H-pyrazole-3-carboxylic acid
3		5-Ethanesulfonylmethyl-1H-pyrazole-3-carboxylic acid
4		5-(2-Oxo-propoxymethyl)-1H-pyrazole-3-carboxylic acid
5		5-Prop-2-ynyloxymethyl-1H-pyrazole-3-carboxylic acid
6		5-Carbamoyl-1H-pyrazole-3-carboxylic acid

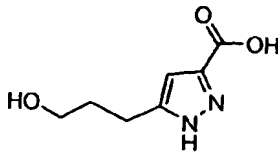
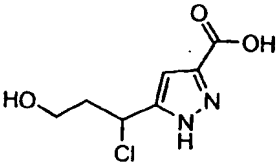
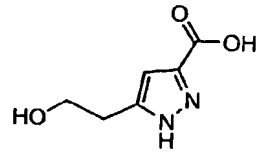
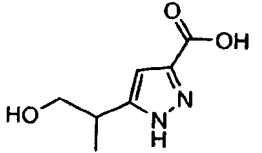
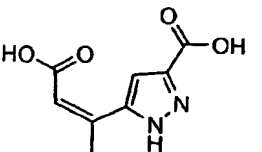
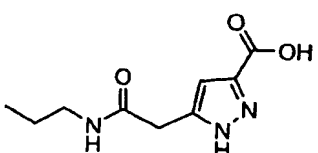
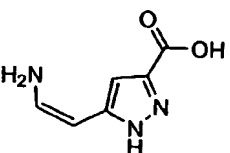
59.US1.PRO

## Provisional Application

Cmpd#	Structure	Chemical Name
7		5-(1-Methylsulfanyl-ethyl)-1H-pyrazole-3-carboxylic acid
8		5-(1-Methanesulfinyl-ethyl)-1H-pyrazole-3-carboxylic acid
9		5-(1-Methanesulfonyl-ethyl)-1H-pyrazole-3-carboxylic acid
10		5-(1,1-Dimethoxy-ethyl)-1H-pyrazole-3-carboxylic acid
11		5-(2-Carboxy-1,1-dimethyl-ethyl)-1H-pyrazole-3-carboxylic acid
12		5-(1-Acetoxy-ethyl)-1H-pyrazole-3-carboxylic acid

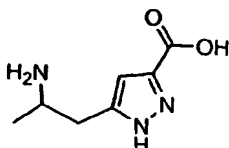
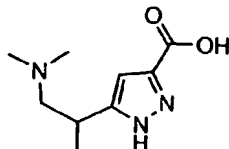
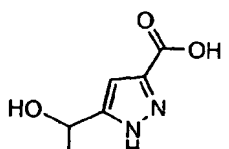
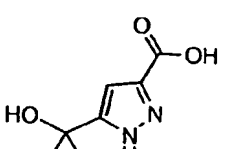
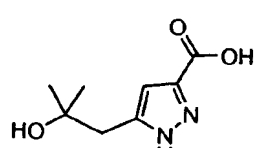
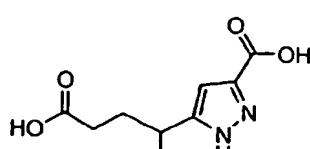
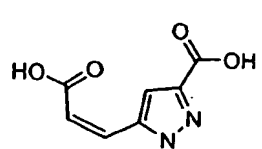
59.US1.PRO

## Provisional Application

Cmpd#	Structure	Chemical Name
13		5-(3-Hydroxy-propyl)-1H-pyrazole-3-carboxylic acid
14		5-(1-Chloro-3-hydroxy-propyl)-1H-pyrazole-3-carboxylic acid
15		5-(2-Hydroxy-ethyl)-1H-pyrazole-3-carboxylic acid
16		5-(2-Hydroxy-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid
17		5-(2-Carboxy-1-methyl-vinyl)-1H-pyrazole-3-carboxylic acid
18		5-Propylcarbamoylmethyl-1H-pyrazole-3-carboxylic acid
19		5-(2-Amino-vinyl)-1H-pyrazole-3-carboxylic acid

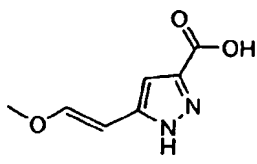
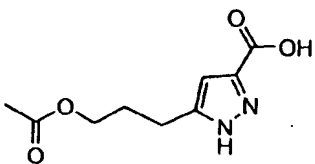
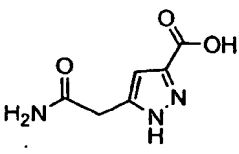
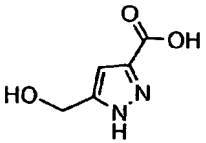
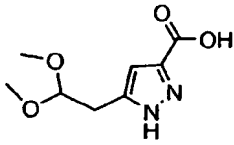
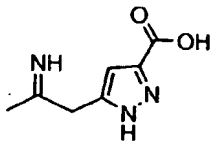
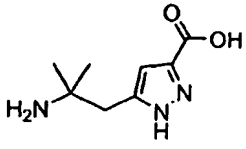
59.US1.PRO

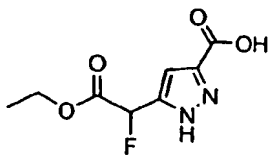
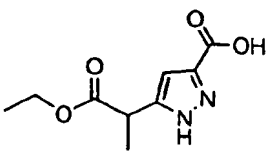
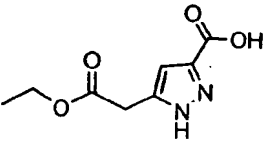
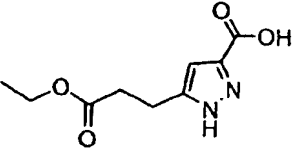
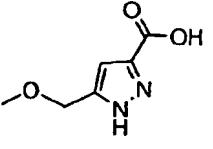
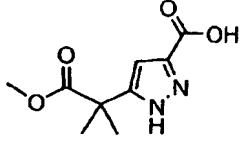
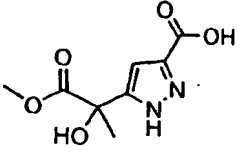
## Provisional Application

Cmpd#	Structure	Chemical Name
20		5-(2-Amino-propyl)-1H-pyrazole-3-carboxylic acid
21		5-(2-Dimethylamino-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid
22		5-(1-Hydroxy-ethyl)-1H-pyrazole-3-carboxylic acid
23		5-(1-Hydroxy-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid
24		5-(2-Hydroxy-2-methyl-propyl)-1H-pyrazole-3-carboxylic acid
25		5-(3-Carboxy-1-methyl-propyl)-1H-pyrazole-3-carboxylic acid
26		5-(2-Carboxy-vinyl)-1H-pyrazole-3-carboxylic acid

59.US1.PRO

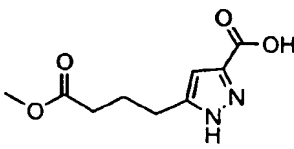
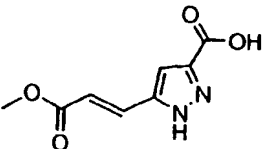
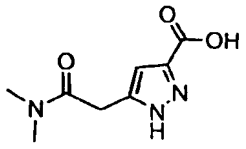
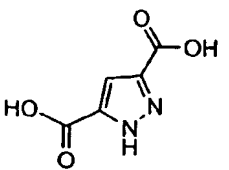
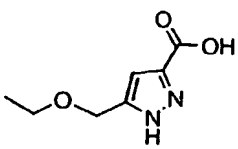
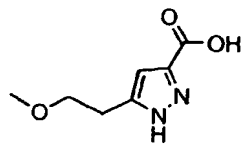
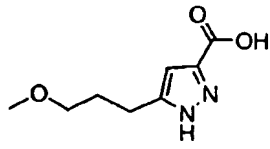
## Provisional Application

Cmpd#	Structure	Chemical Name
27		5-(2-Methoxy-vinyl)-1H-pyrazole-3-carboxylic acid
28		5-(3-Acetoxy-propyl)-1H-pyrazole-3-carboxylic acid
29		5-Carbamoylmethyl-1H-pyrazole-3-carboxylic acid
30		5-Hydroxymethyl-1H-pyrazole-3-carboxylic acid
31		5-(2,2-Dimethoxy-ethyl)-1H-pyrazole-3-carboxylic acid
32		5-(2-Imino-propyl)-1H-pyrazole-3-carboxylic acid
33		5-(2-Amino-2-methyl-propyl)-1H-pyrazole-3-carboxylic acid

Cmpd#	Structure	Chemical Name
34		5-(Ethoxycarbonyl-fluoromethyl)-1H-pyrazole-3-carboxylic acid
35		5-(1-Ethoxycarbonyl-ethyl)-1H-pyrazole-3-carboxylic acid
36		5-Ethoxycarbonylmethyl-1H-pyrazole-3-carboxylic acid
37		5-(2-Ethoxycarbonyl-ethyl)-1H-pyrazole-3-carboxylic acid
38		5-Methoxymethyl-1H-pyrazole-3-carboxylic acid
39		5-(1-Methoxycarbonyl-1-methyl-ethyl)-1H-pyrazole-3-carboxylic acid
40		5-(1-Hydroxy-1-methoxycarbonyl-ethyl)-1H-pyrazole-3-carboxylic acid

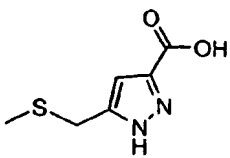
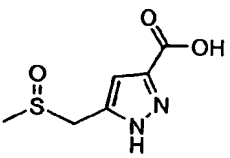
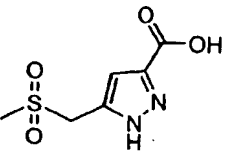
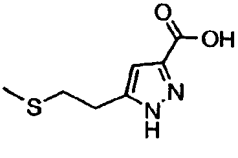
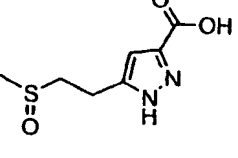
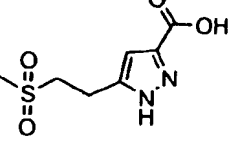
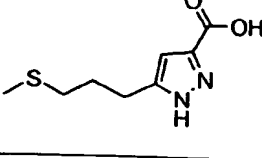
59.US1.PRO

## Provisional Application

Cmpd#	Structure	Chemical Name
41		5-(3-Methoxycarbonyl-propyl)-1H-pyrazole-3-carboxylic acid
42		5-(2-Methoxycarbonyl-vinyl)-1H-pyrazole-3-carboxylic acid
43		5-Dimethylcarbamoylmethyl-1H-pyrazole-3-carboxylic acid
44		1H-Pyrazole-3,5-dicarboxylic acid
45		5-Ethoxymethyl-1H-pyrazole-3-carboxylic acid
46		5-(2-Methoxy-ethyl)-1H-pyrazole-3-carboxylic acid
47		5-(3-Methoxy-propyl)-1H-pyrazole-3-carboxylic acid

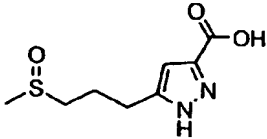
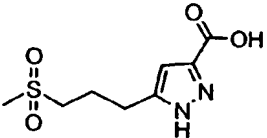
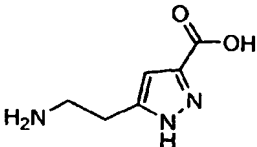
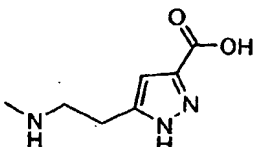
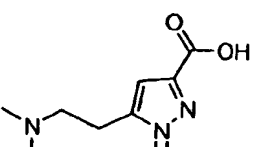
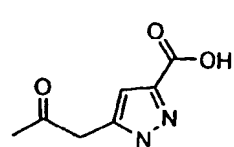
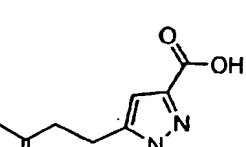
59.US1.PRO

## Provisional Application

Cmpd#	Structure	Chemical Name
48		5-Methylsulfanylmethyl-1H-pyrazole-3-carboxylic acid
49		5-Methanesulfinylmethyl-1H-pyrazole-3-carboxylic acid
50		5-Methanesulfonylmethyl-1H-pyrazole-3-carboxylic acid
51		5-(2-Methylsulfanyl-ethyl)-1H-pyrazole-3-carboxylic acid
52		5-(2-Methanesulfinyl-ethyl)-1H-pyrazole-3-carboxylic acid
53		5-(2-Methanesulfonyl-ethyl)-1H-pyrazole-3-carboxylic acid
54		5-(3-Methylsulfanyl-propyl)-1H-pyrazole-3-carboxylic acid

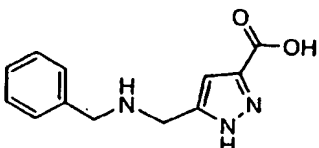
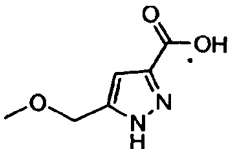
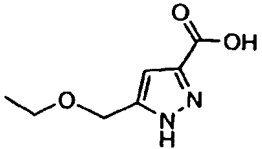
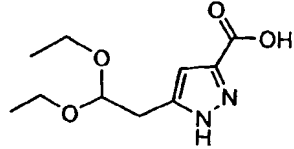
59.US1.PRO

## Provisional Application

Cmpd#	Structure	Chemical Name
55		5-(3-Methanesulfinyl-propyl)-1H-pyrazole-3-carboxylic acid
56		5-(3-Methanesulfonyl-propyl)-1H-pyrazole-3-carboxylic acid
57		5-(2-Amino-ethyl)-1H-pyrazole-3-carboxylic acid
58		5-(2-Methylamino-ethyl)-1H-pyrazole-3-carboxylic acid
59		5-(2-Dimethylamino-ethyl)-1H-pyrazole-3-carboxylic acid
60		5-(2-Oxo-propyl)-1H-pyrazole-3-carboxylic acid
61		5-(3-Oxo-butyl)-1H-pyrazole-3-carboxylic acid

59.US1.PRO

## Provisional Application

Cmpd#	Structure	Chemical Name
62		5-(Benzylamino-methyl)-1H-pyrazole-3-carboxylic acid
63		5-Methoxymethyl-1H-pyrazole-3-carboxylic acid
64		5-Ethoxymethyl-1H-pyrazole-3-carboxylic acid
65		5-(2,2-Diethoxy-ethyl)-1H-pyrazole-3-carboxylic acid

It is understood that the present invention includes compounds shown in TABLE A and corresponding tautomers and esters thereof.

### 5 Compositions of the Present Invention

Some embodiments of the present invention include a method of producing a pharmaceutical composition comprising admixing at least one compound according to any of the compound embodiments disclosed herein and a pharmaceutically acceptable carrier.

10 Formulations can be prepared by any suitable method, typically by uniformly mixing the active compound(s) with liquids or finely divided solid carriers, or both, in the required proportions, and then, if necessary, forming the resulting mixture into a desired shape.

15 Conventional excipients, such as binding agents, fillers, acceptable wetting agents, tableting lubricants, and disintegrants can be used in tablets and capsules for oral

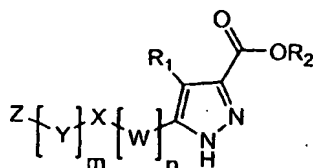
S9.US1.PRO

## Provisional Application

administration. Liquid preparations for oral administration can be in the form of solutions, emulsions, aqueous or oily suspensions, and syrups. Alternatively, the oral preparations can be in the form of dry powder that can be reconstituted with water or another suitable liquid vehicle before use. Additional additives such as suspending or emulsifying agents, non-aqueous vehicles (including edible oils), preservatives, and flavorings and colorants can be added to the liquid preparations. Parenteral dosage forms can be prepared by dissolving the compound of the invention in a suitable liquid vehicle and filter sterilizing the solution before filling and sealing an appropriate vial or ampoule. These are just a few examples of the many appropriate methods well known in the art for preparing dosage forms.

A compound of the present invention can be formulated into pharmaceutical compositions using techniques well known to those in the art. Suitable pharmaceutically-acceptable carriers, outside those mentioned herein, are known in the art; for example, see Remington, The Science and Practice of Pharmacy, 20<sup>th</sup> Edition, 2000, Lippincott Williams & Wilkins, (Editors: Gennaro, A. R., et al.).

While it is possible that a compound for use in the prophylaxis or treatment of the present invention may, in an alternative use, be administered as a raw or pure chemical, it is preferable however to present the compound or "active ingredient" as a pharmaceutical formulation or composition further comprising a pharmaceutically acceptable carrier. Therefore, one aspect of the present invention encompasses pharmaceutical compositions comprising a pharmaceutically acceptable carrier in combination with at least one compound according to Formula (I):



(I)

wherein:

W and Y are independently a straight or branched chain C<sub>1-5</sub> alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said C<sub>1-5</sub> alkylene group is optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> haloalkyl or C<sub>1-4</sub> alkoxy;

X is -NR<sub>3</sub>C(O)-, -C(O)NR<sub>3</sub>, -NR<sub>3</sub>S(O)<sub>2</sub>-, -S(O)<sub>2</sub>NR<sub>3</sub>-,

59.US1.PRO

## Provisional Application

-NR<sub>3</sub>C(O)NR<sub>4</sub>-, -NR<sub>3</sub>C(O)O-, -OC(O)NR<sub>3</sub>-, -NR<sub>3</sub>-, -C(O)-, -CH(OH)-, -C(NH)-, -O-, -S-, -S(O)- or -S(O)<sub>2</sub>-;

R<sub>3</sub> and R<sub>4</sub> are independently H, C<sub>1-4</sub> alkyl, phenyl or heteroaryl, wherein each of said alkyl, phenyl or heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxyl, thiol, cyano, nitro, C<sub>1-4</sub> haloalkyl, amino, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

Z is H, halogen, phenyl or heteroaryl, wherein said phenyl and heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxy, thiol, cyano, nitro, C<sub>1-4</sub> haloalkyl, amino, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

R<sub>1</sub> is H, hydroxyl, halogen, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> haloalkyl;

R<sub>2</sub> is H or C<sub>1-8</sub> alkyl and

"n" and "m" or independently 0 or 1; or

a pharmaceutically acceptable salt, solvate or hydrate thereof;

provided that when X is -NR<sub>3</sub>- then "n" is not 0.

Applicant reserves the right to exclude one or more of the compounds from any pharmaceutical composition embodiment; for example, one or more compounds can be excluded from any pharmaceutical composition embodiment selected from the group consisting of:

i) R<sub>1</sub> and R<sub>2</sub> are both H and -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is selected from the group consisting of: CO<sub>2</sub>H, C(O)-C<sub>6</sub>H<sub>4</sub>-p-O-C<sub>8</sub>H<sub>17</sub>, OCH<sub>2</sub>CH<sub>3</sub>, OH, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H, CH<sub>2</sub>CO<sub>2</sub>H and CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H;

ii) R<sub>1</sub> is CH<sub>3</sub>, R<sub>2</sub> is H and -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is selected from the group consisting of CH<sub>2</sub>CO<sub>2</sub>H, C(O)CH=CH C<sub>6</sub>H<sub>5</sub>, C(O)C<sub>6</sub>H<sub>4</sub>-p-OCH<sub>3</sub>, CO<sub>2</sub>H, C(O)CH<sub>3</sub>, C(O)C<sub>6</sub>H<sub>4</sub>-o-CH<sub>3</sub>, C(O)C<sub>6</sub>H<sub>4</sub>-o-Br, C(O)C<sub>6</sub>H<sub>4</sub>-o-Cl, and C(O)C<sub>6</sub>H<sub>5</sub>;

iii) R<sub>1</sub> is Br, R<sub>2</sub> is H and -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is CO<sub>2</sub>H;

iv) R<sub>1</sub> is OH, R<sub>2</sub> is H and -[W]<sub>n</sub>-X-[Y]<sub>m</sub>-Z together is CO<sub>2</sub>H;

## 59.US1.PRO

## Provisional Application

v)  $R_1$  is H,  $R_2$  is  $CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is selected from the group consisting of 2,6-dichloro-4-trifluoromethylphenoxy,  $C(O)NH-C_6H_4-p-OCH_2CH_3$ ,  $NHC(O)CH(CH_3)_2$ ,  $SCH_3$ ,  $C(O)-C_6H_4-p-O-C_8H_{17}$ ,  $SCH_2CH_3$ ,  $C(O)NHC_6H_5$ ,  $CH(OC_6H_5)_2$ ,  $CH_2OC(O)CH_3$ ,  $CO_2H$ ,  $CO_2CH_3$ ,  $C(O)C_6H_4-p-NO_2$ ,  $C(O)C_6H_5$ ,  $CH_2CH_2CO_2CH_3$ ,  $CH_2CH_2CH_2CH_2CO_2CH_3$ ,  $CH_2CH_2CH_2CO_2CH_3$  and  $CH_2CO_2CH_3$ ;

vi)  $R_1$  is OH,  $R_2$  is  $CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is selected from the group consisting of  $CH_2OCH_2C_6H_5$ ,  $CH_2OCH(CH_3)_2$  and  $CH_2OH$ ;

vii)  $R_2$  is  $CH_3$ :

$R_1$  is  $CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is 2,6-dichloro-4-trifluoromethylphenoxy;

$R_1$  is I and  $-[W]_n-X-[Y]_m-Z$  together is  $CO_2C(CH_3)_3$ ;

$R_1$  is  $C(CH_3)_3$  and  $-[W]_n-X-[Y]_m-Z$  together is formyl;

$R_1$  is Br and  $-[W]_n-X-[Y]_m-Z$  together is  $CO_2CH_3$ ;

and

$R_1$  is  $CH_2CH_2CH_2CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is formyl;

viii)  $R_1$  is H;  $R_2$  is  $CH_2CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is selected from the group consisting of  $CH_2SCH_2CH_3$ ,  $OCH_2CH_2CH=CH_2$ ,  $CH_2CH_2CH_2OH$ ,  $CH_2CH_2CHO$ ,  $CO_2CH_2CH_3$ ,  $OCH_3$ ,  $C(O)CH_2Br$ ,  $CO_2C_8H_{17}$ , formyl, OH,  $CH_2N(CH_2CH_2Cl)_2$ ,  $CH(CH_3)OC(O)CH_3$ ,  $CH_2OH$ ,  $CH_2OC(O)CH_3$ ,  $C(O)CH_3$ ,  $C(O)C_6H_5$  and  $C(O)NHCH_2CO_2CH_2CH_3$ .

ix)  $R_1$  is  $CH_3$ ,  $R_2$  is  $CH_2CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is selected from the group consisting of  $CH(OH)C_6H_4-p-N(CH_3)_2$ ,  $C(O)CH_2C(O)CH_3$ ,  $CO_2CH_2C_6H_5$ ,  $CO_2CH_3$ ,  $C(O)CH_2CH_2CH_3$ ,  $C(O)CH_3$ ,  $C(O)C_6H_4-p-OCH_3$ ,  $C(O)C_6H_4-o-Br$ ,  $C(O)C_6H_4-p-Cl$ ,  $C(O)C_6H_4-o-Cl$ ,  $C(O)CH_2C_6H_5$  and  $C(O)C_6H_5$ ;

x)  $R_2$  is  $CH_2CH_3$ :

$R_1$  is I and  $-[W]_n-X-[Y]_m-Z$  together is  $CO_2CH_2CH_3$ ;

$R_1$  is  $CF_3$  and  $-[W]_n-X-[Y]_m-Z$  together is  $CO_2CH_2CH_3$ ;

and

$R_1$  is Br and  $-[W]_n-X-[Y]_m-Z$  together is  $CO_2CH_2CH_3$ ;

xi)  $R_1$  is OH,  $R_2$  is  $CH_2CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is selected from the group consisting of  $C(O)C_6H_5$ ,  $C(O)NH_2$  and  $CO_2CH_2CH_3$ ;

xii)  $R_1$  is H,  $R_2$  is  $C(CH_3)_3$  and  $-[W]_n-X-[Y]_m-Z$  together is selected from the group consisting of  $CO_2C(CH_3)_3$ ,  $C(O)NHC(O)CH_3$  and  $C(O)NH_2$ ; and

xiii)  $R_1$  is OH,  $R_2$  is  $CH_2CH_2CH_2CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is  $C(O)C_6H_5$ .

5 While it is possible that a compound of the invention may, in an alternative mode, be administered as a raw or pure chemical, it is preferable to present the compound or "active ingredient" as a pharmaceutical formulation or composition.

10 The invention provides pharmaceutical formulations comprising a compound of the invention or a pharmaceutically acceptable salt or derivative thereof together with one or more pharmaceutically acceptable carriers therefor. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not overly deleterious to the recipient thereof.

15 Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, subcutaneous and intravenous) administration or in a form suitable for administration by inhalation, insufflation or by a transdermal patch. Transdermal patches dispense a drug at a controlled rate by presenting the drug for absorption in an efficient manner with a minimum of degradation of the drug. Typically, transdermal patches comprise an impermeable backing layer, a single pressure sensitive adhesive and a removable protective layer with a release liner. One of ordinary skill in the art will understand and appreciate the techniques appropriate for manufacturing a desired efficacious transdermal patch based upon the needs of the artisan.

20 The compounds of the invention, together with a conventional adjuvant, carrier, or diluent, may thus be placed into the form of pharmaceutical formulations and unit dosages thereof, and in such form can be employed as solids, such as tablets or filled capsules, or liquids such as solutions, suspensions, emulsions, elixirs, gels or capsules filled with the same, all for oral use, in the form of suppositories for rectal administration; or in the form of sterile injectable solutions for parenteral (including subcutaneous) use. Such pharmaceutical compositions and unit dosage forms thereof may comprise conventional ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed.

59.US1.PRO

Provisional Application

For oral administration, the pharmaceutical composition can be in the form of, for example, a tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are capsules, tablets, powders, granules or a suspension, with conventional additives such as lactose, mannitol, corn starch or potato starch; with binders such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators such as corn starch, potato starch or sodium carboxymethyl-cellulose; and with lubricants such as talc or magnesium stearate. The active ingredient may also be administered by injection as a composition wherein, for example, saline, dextrose or water can be used as a suitable pharmaceutically acceptable carrier.

Compounds of the present invention or a solvate or physiologically functional derivative thereof can be used as active ingredients in pharmaceutical compositions, specifically as RUP25 receptor agonists. By the term "active ingredient" is defined in the context of a "pharmaceutical composition" and shall mean a component of a pharmaceutical composition that provides the primary pharmacological effect, as opposed to an "inactive ingredient" which would generally be recognized as providing no pharmaceutical benefit.

The dose when using the compounds of the present invention can vary within wide limits, and as is customary and is known to the physician, it is to be tailored to the individual conditions in each individual case. It depends, for example, on the nature and severity of the illness to be treated, on the condition of the patient, on the compound employed or on whether an acute or chronic disease state is treated or prophylaxis is conducted or on whether further active compounds are administered in addition to the compounds of the present invention. Representative doses of the present invention include, but not limited to, about 0.001 mg to about 5000 mg, about 0.001 to about 2500 mg, about 0.001 to about 1000 mg, 0.001 to about 500 mg, 0.001 mg to about 250 mg, about 0.001 mg to 100 mg, about 0.001 mg to about 50 mg, and about 0.001 mg to about 25 mg. Multiple doses can be administered during the day, especially when relatively large amounts are deemed to be needed, for example 2, 3 or 4, doses. Depending on the individual and as deemed appropriate from the patient's physician or care-giver it may be necessary to deviate upward or downward from the doses described herein.

The amount of active ingredient, or an active salt or derivative thereof, required for use in prophylaxis or treatment will vary not only with the particular salt selected but

59.US1.PRO

## Provisional Application

also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will ultimately be at the discretion of the attendant physician or clinician. In general, one skilled in the art understands how to extrapolate *in vivo* data obtained in a model system to another, for example, an animal model to a human. Typically, animal models include, but are not limited to, the rodents diabetes models as described in Example 15, *infra*; the mouse atherosclerosis model as described in Example 16, *infra*; or the *in vivo* animal atherosclerosis model as described in Example 17, *infra*. In some circumstances, these extrapolations may merely be based on the weight of the animal model in comparison to another, such as a mammal, preferably a human, however, more often, these extrapolations are not simply based on weight differences, but rather incorporate a variety of factors. Representative factors include the type, age, weight, sex, diet and medical condition of the patient, the severity of the disease, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized, on whether an acute or chronic disease state is being treated or prophylaxis is conducted or on whether further active compounds are administered in addition to the compounds of the Formula (I) and as part of a drug combination. The dosage regimen for treating a disease condition with the compounds and/or compositions of this invention is selected in accordance with a variety factors, such as, those cited above. Thus, the actual dosage regimen employed may vary widely and therefore may deviate from a preferred dosage regimen and one skilled in the art will recognize that dosage and dosage regimen outside these typical ranges can be tested and, where appropriate, can be used in the methods of this invention.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself can be further divided, e.g., into a number of discrete loosely spaced administrations. The daily dose can be divided, especially when relatively large amounts are administered as deemed appropriate, into several, for example 2, 3 or 4, part administrations. If appropriate, depending on individual behavior, it can be necessary to deviate upward or downward from the daily dose indicated.

The compounds of the present invention can be administered in a wide variety of oral and parenteral dosage forms. It will be obvious to those skilled in the art that the

59.US1.PRO

## Provisional Application

following dosage forms may comprise, as the active component, either a compound of the invention or a pharmaceutically acceptable salt of a compound of the invention.

For preparing pharmaceutical compositions from the compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavouring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component.

In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted to the desired shape and size.

The powders and tablets may contain varying percentage amounts of the active compound. A representative amount in a powder or tablet may contain from 0.5 to about 90 percent of the active compound; however, an artisan would know when amounts outside of this range are necessary. Suitable carriers for powders and tablets are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the formulation of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid forms suitable for oral administration.

For preparing suppositories, a low melting wax, such as an admixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water-propylene glycol solutions. For example, parenteral injection liquid preparations can be formulated as solutions in aqueous polyethylene glycol

## 59.US1.PRO

## Provisional Application

5 solution. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

10 The compounds according to the present invention may thus be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and can be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

20 Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavours, stabilizing and thickening agents, as desired.

25 Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, or other well known suspending agents.

30 Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

For topical administration to the epidermis the compounds according to the invention can be formulated as ointments, creams or lotions, or as a transdermal patch.

59.US1.PRO

## Provisional Application

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions can be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active agent in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations can be provided in single or multi-dose form. In the latter case of a dropper or pipette, this can be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray, this can be achieved for example by means of a metering atomizing spray pump.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the active ingredient is provided in a pressurized pack with a suitable propellant. If the compounds of the Formula (I) or pharmaceutical compositions comprising them are administered as aerosols, for example as nasal aerosols or by inhalation, this can be carried out, for example, using a spray, a nebulizer, a pump nebulizer, an inhalation apparatus, a metered inhaler or a dry powder inhaler. Pharmaceutical forms for administration of the compounds of the Formula (I) as an aerosol can be prepared by processes well-known to the person skilled in the art. For their preparation, for example, solutions or dispersions of the compounds of the Formula (I) in water, water/alcohol mixtures or suitable saline solutions can be employed using customary additives, for example benzyl alcohol or other suitable preservatives, absorption enhancers for increasing the bioavailability, solubilizers, dispersants and others, and, if appropriate, customary propellants, for example include carbon dioxide, CFC's, such as, dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane; and the like. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug can be controlled by provision of a metered valve.

## 59.US1.PRO

## Provisional Application

In formulations intended for administration to the respiratory tract, including intranasal formulations, the compound will generally have a small particle size for example of the order of 10 microns or less. Such a particle size can be obtained by means known in the art, for example by micronization. When desired, formulations adapted to give sustained release of the active ingredient can be employed.

Alternatively the active ingredients can be provided in the form of a dry powder, for example, a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition can be presented in unit dose form for example in capsules or cartridges of, e.g., gelatin, or blister packs from which the powder can be administered by means of an inhaler.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

Tablets or capsules for oral administration and liquids for intravenous administration are preferred compositions.

Compounds of the present invention can be converted to "pro-drugs." The term "pro-drugs" refers to compounds that have been modified with specific chemical groups known in the art and when administered into an individual these groups undergo biotransformation to give the parent compound. Pro-drugs can thus be viewed as compounds of the invention containing one or more specialized non-toxic protective groups used in a transient manner to alter or to eliminate a property of the compound. In general, the "pro-drug" approach is utilized to facilitate oral absorption. A thorough discussion is provided in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference in their entirety.

59.US1.PRO

## Provisional Application

**Combination Therapy - Prophylaxis and Treatment:**

While the compounds of the present invention can be administered as the sole active pharmaceutical agent (i.e., mono-therapy), they can also be used in combination with other pharmaceutical agents (i.e., combination-therapy), such as, for the treatment of the diseases/conditions/disorders described herein. Therefore, another aspect of the present invention includes methods of prophylaxis and/or treatment of metabolic related diseases comprising administering to an individual in need of such prophylaxis and/or treatment a therapeutically effective amount of a compound of the present invention in combination with one or more additional pharmaceutical agent as described herein.

Suitable pharmaceutical agents that can be used in combination with the compounds of the present invention include anti-obesity agents such as apolipoprotein-B secretion/microsomal triglyceride transfer protein (apo-B/MTP) inhibitors, MCR-4 agonists, cholecystokinin-A (CCK-A) agonists, serotonin and norepinephrine reuptake inhibitors (for example, sibutramine), sympathomimetic agents,  $\beta_3$  adrenergic receptor agonists, dopamine agonists (for example, bromocriptine), melanocyte-stimulating hormone receptor analogs, cannabinoid 1 receptor antagonists [for example, SR141716: *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide], melanin concentrating hormone antagonists, leptons (the OB protein), leptin analogues, leptin receptor agonists, galanin antagonists, lipase inhibitors (such as tetrahydrolipstatin, i.e., Orlistat), anorectic agents (such as a bombesin agonist), Neuropeptide-Y antagonists, thyromimetic agents, dehydroepiandrosterone or an analogue thereof, glucocorticoid receptor agonists or antagonists, orexin receptor antagonists, urocortin binding protein antagonists, glucagon-like peptide-1 receptor agonists, ciliary neurotrophic factors (such as Axokine™ available from Regeneron Pharmaceuticals, Inc., Tarrytown, NY and Procter & Gamble Company, Cincinnati, OH), human agouti-related proteins (AGRP), ghrelin receptor antagonists, histamine 3 receptor antagonists or reverse agonists, neuromedin U receptor agonists, noradrenergic anorectic agents (for example, phentermine, mazindol and the like) and appetite suppressants (for example, bupropion).

Other anti-obesity agents, including the agents set forth *infra*, are well known, or will be readily apparent in light of the instant disclosure, to one of ordinary skill in the art.

59.US1.PRO

Provisional Application

In some embodiments, the anti-obesity agents are selected from the group consisting of orlistat, sibutramine, bromocriptine, ephedrine, leptin, and pseudoephedrine. In a further embodiment, compounds of the present invention and combination therapies are administered in conjunction with exercise and/or a sensible diet.

It is understood that the scope of combination-therapy of the compounds of the present invention with other anti-obesity agents, anorectic agents, appetite suppressant and related agents is not limited to those listed above, but includes in principle any combination with any pharmaceutical agent or pharmaceutical composition useful for the treatment of overweight and obese individuals.

Other suitable pharmaceutical agents, in addition to anti-obesity agents, that can be used in combination with the compounds of the present invention include agents useful in the prophylaxis or treatment of concomitant disorders. Treatment of such disorders include the use of one or more pharmaceutical agents known in the art that belong to the classes of drugs referred to, but not limited to, the following: sulfonylureas, meglitinides, biguanides,  $\alpha$ -glucosidase inhibitors, peroxisome proliferators-activated receptor- $\gamma$  (i.e., PPAR- $\gamma$ ) agonists, insulin, insulin analogues, HMG-CoA reductase inhibitors, cholesterol-lowering drugs (for example, fibrates that include: fenofibrate, bezafibrate, gemfibrozil, clofibrate and the like; bile acid sequestrants which include: cholestyramine, colestipol and the like; and niacin), antiplatelet agents (for example, aspirin and adenosine diphosphate receptor antagonists that include: clopidogrel, ticlopidine and the like), angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists and adiponectin. In accordance to one aspect of the present invention, a compound of the present can be used in combination with a pharmaceutical agent or agents belonging to one or more of the classes of drugs cited herein.

It is understood that the scope of combination-therapy of the compounds of the present invention with other pharmaceutical agents is not limited to those listed herein, *supra* or *infra*, but includes in principle any combination with any pharmaceutical agent or pharmaceutical composition useful for the treatment of diseases, conditions or disorders that are linked to metabolic-related disorders.

Some embodiments of the present invention include methods of prophylaxis or treatment of a disease, disorder or condition as described herein comprising administering to an individual in need of such prophylaxis or treatment a therapeutically

59.US1.PRO

## Provisional Application

effect amount or dose of a compound of the present invention in combination with at least one pharmaceutical agent selected from the group consisting of: sulfonylureas, meglitinides, biguanides,  $\alpha$ -glucosidase inhibitors, peroxisome proliferators-activated receptor- $\gamma$  (i.e., PPAR- $\gamma$ ) agonists, insulin, insulin analogues, HMG-CoA reductase inhibitors, cholesterol-lowering drugs (for example, fibrates that include: fenofibrate, bezafibrate, gemfibrozil, clofibrate and the like; bile acid sequestrants which include: cholestyramine, colestipol and the like; and niacin), antiplatelet agents (for example, aspirin and adenosine diphosphate receptor antagonists that include: clopidogrel, ticlopidine and the like), angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists and adiponectin. In some embodiments, the pharmaceutical composition further comprises one or more agents selected from the group consisting of  $\alpha$ -glucosidase inhibitor, aldose reductase inhibitor, biguanide, HMG-CoA reductase inhibitor, squalene synthesis inhibitor, fibrate, LDL catabolism enhancer, angiotensin converting enzyme inhibitor, insulin secretion enhancer and thiazolidinedione.

One aspect of the present invention encompasses pharmaceutical compositions comprising at least one compound according to Formula (I), as described herein. In some embodiments, the pharmaceutical composition further comprises one or more agents selected from the group consisting of, for example,  $\alpha$ -glucosidase inhibitor, aldose reductase inhibitor, biguanide, HMG-CoA reductase inhibitor, squalene synthesis inhibitor, fibrate, LDL catabolism enhancer, angiotensin converting enzyme inhibitor, insulin secretion enhancer and thiazolidinedione.

Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include  $\alpha$ -glucosidase inhibitors.  $\alpha$ -Glucosidase inhibitors belong to the class of drugs which competitively inhibit digestive enzymes such as  $\alpha$ -amylase, maltase,  $\alpha$ -dextrinase, sucrase, etc. in the pancreas and or small intestine. The reversible inhibition by  $\alpha$ -glucosidase inhibitors retard, diminish or otherwise reduce blood glucose levels by delaying the digestion of starch and sugars. Some representative examples of  $\alpha$ -glucosidase inhibitors include acarbose, N-(1,3-dihydroxy-2-propyl)valiolamine (generic name; voglibose), miglitol, and  $\alpha$ -glucosidase inhibitors known in the art.

Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include sulfonylureas. The sulfonylureas (SU) are drugs which promote secretion of insulin from pancreatic  $\beta$  cells by transmitting signals of insulin

## 59.US1.PRO

## Provisional Application

secretion via SU receptors in the cell membranes. Examples of the sulfonylureas include glyburide, glipizide, glimepiride and other sulfonylureas known in the art.

5        Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include the meglitinides. The meglitinides are benzoic acid derivatives represent a novel class of insulin secretagogues. These agents target postprandial hyperglycemia and show comparable efficacy to sulfonylureas in reducing HbA<sub>1c</sub>. Examples of meglitinides include repaglinide, nateglinide and other meglitinides known in the art.

10        Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include the biguanides. The biguanides represent a class of drugs that stimulate anaerobic glycolysis, increase the sensitivity to insulin in the peripheral tissues, inhibit glucose absorption from the intestine, suppress of hepatic gluconeogenesis, and inhibit fatty acid oxidation. Examples of biguanides include phenformin, metformin, buformin, and biguanides known in the art.

15        Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include the  $\alpha$ -glucosidase inhibitors. The  $\alpha$ -glucosidase inhibitors competitively inhibit digestive enzymes such as  $\alpha$ -amylase, maltase,  $\alpha$ -dextrinase, sucrase, etc. in the pancreas and or small intestine. The reversible inhibition by  $\alpha$ -glucosidase inhibitors retard, diminish or otherwise reduce blood glucose levels by  
20        delaying the digestion of starch and sugars. Examples of  $\alpha$ -glucosidase inhibitors include acarbose, N-(1,3-dihydroxy-2-propyl)valiolaminc (generic name; voglibose), miglitol, and  $\alpha$ -glucosidase inhibitors known in the art.

25        Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include the peroxisome proliferators-activated receptor- $\gamma$  (i.e., PPAR- $\gamma$ ) agonists. The peroxisome proliferators-activated receptor- $\gamma$  agonists represent a class of compounds that activates the nuclear receptor PPAR- $\gamma$  and therefore regulate the transcription of insulin-responsive genes involved in the control of glucose production, transport and utilization. Agents in the class also facilitate the regulation of fatty acid metabolism. Examples of PPAR- $\gamma$  agonists include rosiglitazone,  
30        pioglitazone, tesaglitazar, netoglitazone, GW-409544, GW-501516 and PPAR- $\gamma$  agonists known in the art.

      Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include the HMG-CoA reductase inhibitors. The HMG-CoA

59.US1.PRO

## Provisional Application

reductase inhibitors are agents also referred to as Statin compounds that belong to a class of drugs that lower blood cholesterol levels by inhibiting hydroxymethylglutaryl CoA (HMG-CoA) reductase. HMG-CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis. The statins lower serum LDL concentrations by upregulating the activity of LDL receptors and are responsible for clearing LDL from the blood. Some representative examples the statin compounds include rosuvastatin, pravastatin and its sodium salt, simvastatin, lovastatin, atorvastatin, fluvastatin, cerivastatin, rosuvastatin, pitavastatin, BMS's "superstatin", and HMG-CoA reductase inhibitors known in the art.

Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include the angiotensin converting enzyme (ACE) inhibitors. The angiotensin converting enzyme inhibitors belong to the class of drugs that partially lower blood glucose levels as well as lowering blood pressure by inhibiting angiotensin converting enzymes. Examples of the angiotensin converting enzyme inhibitors include captopril, enalapril, alacepril, delapril; ramipril, lisinopril, imidapril, benazepril, ceronapril, cilazapril, enalaprilat, fosinopril, moxeltopril, perindopril, quinapril, spirapril, temocapril, trandolapril, and angiotensin converting enzyme inhibitors known in the art.

Suitable pharmaceutical agents that can be used in conjunction with compounds of the present invention include the angiotensin II receptor antagonists. Angiotensin II receptor antagonists target the angiotensin II receptor subtype 1 (i.e., AT1) and demonstrate a beneficial effect on hypertension. Examples of angiotensin II receptor antagonists include losartan (and the potassium salt form), and angiotensin II receptor antagonists known in the art.

Other treatments for one or more of the diseases cited herein include the use of one or more pharmaceutical agents known in the art that belong to the classes of drugs referred to, but not limited to, the following: amylin agonists (for example, pramlintide), insulin secretagogues (for example, GLP-1 agonists; exendin-4; insulinotropin (NN2211); dipeptyl peptidase inhibitors (for example, NVP-DPP-728), acyl CoA cholesterol acetyltransferase inhibitors (for example, Ezetimibe, eflucimibe, and like compounds), cholesterol absorption inhibitors (for example, ezetimibe, pamaqueside and like compounds), cholesterol ester transfer protein inhibitors (for example, CP-529414, JTT-705, CETi-1, and like compounds), microsomal triglyceride transfer protein inhibitors (for example, implitapide, and like compounds), cholesterol modulators (for example, NO-1886, and like compounds), bile acid modulators (for example, GT103-279 and like compounds) and squalene synthase inhibitors.

## 59.US1.PRO

## Provisional Application

Squalene synthesis inhibitors belong to a class of drugs that lower blood cholesterol levels by inhibiting synthesis of squalene. Examples of the squalene synthesis inhibitors include (S)- $\alpha$ -[Bis[2,2-dimethyl-1-oxopropoxy)methoxy] phosphinyl]-3-phenoxybenzenebutanesulfonic acid, mono potassium salt (BMS-188494) and squalene synthesis inhibitors known in the art.

In accordance with the present invention, the combination can be used by mixing the respective active components either all together or independently with a pharmaceutically acceptable carrier, excipient, binder, diluent, etc., as described herein above, and administering the mixture or mixtures either orally or non-orally as a pharmaceutical composition. When a compound or a mixture of compounds of Formula (I) are administered as a combination therapy or prophylaxis with another active compound the therapeutic agents can be formulated as separate pharmaceutical compositions given at the same time or at different times, or the therapeutic agents can be given as a single composition.

In accordance with the present invention, the combination of a compound of the present invention and pharmaceutical agent can be prepared by mixing the respective active components either all together or independently with a pharmaceutically acceptable carrier, excipient, binder, diluent, etc., as described herein, and administering the mixture or mixtures either orally or non-orally as a pharmaceutical composition. When a compound or a mixture of compounds of Formula (I) are administered as a combination therapy or prophylaxis with another active compound the therapeutic agents can be formulated as a separate pharmaceutical compositions given at the same time or at different times, or the therapeutic agents can be given as a single composition.

## 25 Other Utilities

Another object of the present invention relates to radio-labeled compounds of Formula (I) that are useful not only in radio-imaging but also in assays, both in vitro and in vivo, for localizing and quantitating RUP25 in tissue samples, including human, and for identifying RUP25 ligands by inhibition binding of a radio-labeled compound. It is a further object of this invention to include novel RUP25 assays of which comprise such radio-labeled compounds.

The present invention embraces isotopically-labeled compounds of Formula (I) and any subgenera herein, such as but not limited to, Formulae (Ia) to (Iz); (IIa) to (IIy);

## 59.US1.PRO

## Provisional Application

(IIIa) to (IIIc); and (IVa) to (IVc). An "isotopically" or "radio-labeled" compounds are those which are identical to compounds disclosed herein, but for the fact that one or more atoms are replaced or substituted by an atom having an atomic mass or mass number different from the atomic mass or mass number typically found in nature (i.e., naturally occurring). Suitable radionuclides that can be incorporated in compounds of the present invention include but are not limited to  $^2\text{H}$  (also written as D for deuterium),  $^3\text{H}$  (also written as T for tritium),  $^{11}\text{C}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{N}$ ,  $^{15}\text{O}$ ,  $^{17}\text{O}$ ,  $^{18}\text{O}$ ,  $^{18}\text{F}$ ,  $^{35}\text{S}$ ,  $^{36}\text{Cl}$ ,  $^{82}\text{Br}$ ,  $^{75}\text{Br}$ ,  $^{76}\text{Br}$ ,  $^{77}\text{Br}$ ,  $^{123}\text{I}$ ,  $^{124}\text{I}$ ,  $^{125}\text{I}$  and  $^{131}\text{I}$ . The radionuclide that is incorporated in the instant radio-labeled compounds will depend on the specific application of that radio-labeled compound. For example, for *in vitro* RUP25 labeling and competition assays, compounds that incorporate  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{82}\text{Br}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or will generally be most useful. For radio-imaging applications  $^{11}\text{C}$ ,  $^{18}\text{F}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{124}\text{I}$ ,  $^{131}\text{I}$ ,  $^{75}\text{Br}$ ,  $^{76}\text{Br}$  or  $^{77}\text{Br}$  will generally be most useful.

It is understood that a "radio-labeled" or "labeled compound" is a compound of Formula (I) that has incorporated at least one radionuclide; in some embodiments the radionuclide is selected from the group consisting of  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{125}\text{I}$ ,  $^{35}\text{S}$  and  $^{82}\text{Br}$ .

Certain isotopically-labeled compounds of the present invention are useful in compound and/or substrate tissue distribution assays. In some embodiments the radionuclide  $^3\text{H}$  and/or  $^{14}\text{C}$  isotopes are useful in these studies. Further, substitution with heavier isotopes such as deuterium (i.e.,  $^2\text{H}$ ) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased *in vivo* half-life or reduced dosage requirements) and hence can be preferred in some circumstances. Isotopically labeled compounds of the present invention can generally be prepared by following procedures analogous to those disclosed in the Schemes *supra* and Examples *infra*, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent. Other synthetic methods that are useful are discussed *infra*. Moreover, it should be understood that all of the atoms represented in the compounds of the invention can be either the most commonly occurring isotope of such atoms or the more scarce radio-isotope or nonradio-active isotope.

Synthetic methods for incorporating radio-isotopes into organic compounds are applicable to compounds of the invention and are well known in the art. These synthetic methods, for example, incorporating activity levels of tritium into target molecules, and are as follows:

## 59.US1.PRO

## Provisional Application

A. Catalytic Reduction with Tritium Gas - This procedure normally yields high specific activity products and requires halogenated or unsaturated precursors.

B. Reduction with Sodium Borohydride [ $^3\text{H}$ ] - This procedure is rather inexpensive and requires precursors containing reducible functional groups such as aldehydes, ketones, lactones, esters, and the like.

C. Reduction with Lithium Aluminum Hydride [ $^3\text{H}$ ] - This procedure offers products at almost theoretical specific activities. It also requires precursors containing reducible functional groups such as aldehydes, ketones, lactones, esters, and the like.

D. Tritium Gas Exposure Labeling - This procedure involves exposing precursors containing exchangeable protons to tritium gas in the presence of a suitable catalyst.

E. N-Methylation using Methyl Iodide [ $^3\text{H}$ ] - This procedure is usually employed to prepare O-methyl or N-methyl ( $^3\text{H}$ ) products by treating appropriate precursors with high specific activity methyl iodide ( $^3\text{H}$ ). This method in general allows for higher specific activity, such as for example, about 70-90 Ci/mmol.

Synthetic methods for incorporating activity levels of  $^{125}\text{I}$  into target molecules include:

A. Sandmeyer and like reactions - This procedure transforms an aryl or heteroaryl amine into a diazonium salt, such as a tetrafluoroborate salt, and subsequently to  $^{125}\text{I}$  labeled compound using  $\text{Na}^{125}\text{I}$ . A represented procedure was reported by Zhu, D.-G. and co-workers in *J. Org. Chem.* 2002, 67, 943-948.

B. Ortho  $^{125}\text{I}$  iodination of phenols - This procedure allows for the incorporation of  $^{125}\text{I}$  at the ortho position of a phenol as reported by Collier, T. L. and co-workers in *J. Labeled Compd Radiopharm.* 1999, 42, S264-S266.

C. Aryl and heteroaryl bromide exchange with  $^{125}\text{I}$  - This method is generally a two step process. The first step is the conversion of the aryl or heteroaryl bromide to the corresponding tri-alkyltin intermediate using for example, a Pd catalyzed reaction [i.e.  $\text{Pd}(\text{Ph}_3\text{P})_4$ ] or through an aryl or heteroaryl lithium, in the presence of a tri-alkyltinhalide or hexaalkylditin [e.g.,  $(\text{CH}_3)_3\text{SnSn}(\text{CH}_3)_3$ ]. A represented procedure was reported by Bas, M.-D. and co-workers in *J. Labeled Compd Radiopharm.* 2001, 44, S280-S282.

A radio-labeled RUP25 compound of Formula (I) can be used in a screening assay to identify/evaluate compounds. In general terms, a newly synthesized or identified compound (i.e., test compound) can be evaluated for its ability to reduce binding of the "radio-labeled compound of Formula (I)" to the RUP25 receptor.

## 59.US1.PRO

## Provisional Application

Accordingly, the ability of a test compound to compete with the "radio-labeled compound of Formula (I)" for the binding to the RUP25 receptor directly correlates to its binding affinity.

The labeled compounds of the present invention bind to the RUP25 receptor. In one embodiment the labeled compound has an  $IC_{50}$  less than about 500  $\mu M$ , in another embodiment the labeled compound has an  $IC_{50}$  less than about 100  $\mu M$ , in yet another embodiment the labeled compound has an  $IC_{50}$  less than about 10  $\mu M$ , in yet another embodiment the labeled compound has an  $IC_{50}$  less than about 1  $\mu M$ , and in still yet another embodiment the labeled inhibitor has an  $IC_{50}$  less than about 0.1  $\mu M$ .

Other uses of the disclosed receptors and methods will become apparent to those in the art based upon, inter alia, a review of this disclosure.

As will be recognized, the steps of the methods of the present invention need not be performed any particular number of times or in any particular sequence. Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are intended to be illustrative and not intended to be limiting.

## EXAMPLES

The following Examples are provided for illustrative purposes and not as a means of limitation. One of ordinary skill in the art would be able to design equivalent assays and methods based on the disclosure herein, all of which form part of the present invention.

## Example 1

## Full Length Cloning

## hRUP25 (Seq. Id. Nos. 1 &amp; 2)

The disclosed human hRUP25 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC026331 was identified as a human genomic sequence from chromosome 12. The full length hRUP25 was cloned by PCR using primers:  
5'-GCTGGAGCATTCACTAGGCGAG-3' (SEQ.ID.NO.:3; sense, 5' of initiation codon),  
5'-AGATCCTGGTCTTGGTGACAATG-3' (SEQ.ID.NO.:4; antisense, 3' of stop codon)

## 59.US1.PRO

## Provisional Application

and human genomic DNA (Promega) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94°C for 1 minute; 94°C for 15 seconds; 56°C for 20 seconds 72°C for 1 minute 30 seconds and 72°C for 5 minutes.

5 A 1.2kb PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:1 for nucleic acid sequence and SEQ.ID.NO.:2 for deduced amino acid sequence.

## 10 Example 2

## RECEPTOR EXPRESSION

Although a variety of cells are available to the art for the expression of proteins, it is most preferred that mammalian cells be utilized. The primary reason for this is predicated upon practicalities, *i.e.*, utilization of, *e.g.*, yeast cells for the  
15 expression of a GPCR, while possible, introduces into the protocol a non-mammalian cell which may not (indeed, in the case of yeast, does not) include the receptor-coupling, genetic-mechanism and secretory pathways that have evolved for mammalian systems – thus, results obtained in non-mammalian cells, while of potential use, are not as preferred as that obtained from mammalian cells. Of the  
20 mammalian cells, COS-7, 293 and 293T cells are particularly preferred, although the specific mammalian cell utilized can be predicated upon the particular needs of the artisan.

## a. Transient Transfection

On day one,  $6 \times 10^6$  / 10 cm dish of 293 cells well were plated out. On day two,  
25 two reaction tubes were prepared (the proportions to follow for each tube are per plate): tube A was prepared by mixing 4µg DNA (*e.g.*, pCMV vector; pCMV vector with receptor cDNA, etc.) in 0.5 ml serum free DMEM (Gibco BRL); tube B was prepared by mixing 24µl lipofectamine (Gibco BRL) in 0.5ml serum free DMEM. Tubes A and B were admixed by inversions (several times), followed by incubation at room temperature  
30 for 30-45min. The admixture is referred to as the “transfection mixture”. Plated 293 cells were washed with 1XPBS, followed by addition of 5 ml serum free DMEM. 1 ml of the transfection mixture were added to the cells, followed by incubation for 4hrs at 37°C/5% CO<sub>2</sub>. The transfection mixture was removed by aspiration, followed by the

59.US1.PRO

## Provisional Application

addition of 10ml of DMEM/10% Fetal Bovine Serum. Cells were incubated at 37°C/5% CO<sub>2</sub>. After 48hr incubation, cells were harvested and utilized for analysis.

**b. Stable Cell Lines: Gs Fusion Protein**

Approximately  $12 \times 10^6$  293 cells are plated on a 15cm tissue culture plate.

5 Grown in DME High Glucose Medium containing ten percent fetal bovine serum and one percent sodium pyruvate, L-glutamine, and anti-biotics. Twenty-four hours following plating of 293 cells (or to ~80% confluency), the cells are transfected using 12µg of DNA. The 12µg of DNA is combined with 60µl of lipofectamine and 2mL of DME High Glucose Medium without serum. The medium is aspirated from the plates and the cells are washed once with medium without serum. The DNA, lipofectamine, and medium mixture are added to the plate along with 10mL of medium without serum. 10 Following incubation at 37 degrees Celsius for four to five hours, the medium is aspirated and 25ml of medium containing serum is added. Twenty-four hours following transfection, the medium is aspirated again, and fresh medium with serum is added. 15 Forty-eight hours following transfection, the medium is aspirated and medium with serum is added containing geneticin (G418 drug) at a final concentration of 500µg/mL. The transfected cells now undergo selection for positively transfected cells containing the G418 resistant gene. The medium is replaced every four to five days as selection occurs. During selection, cells are grown to create stable pools, or split for stable clonal selection. 20

**Example 3**

**ASSAYS FOR DETERMINATION OF CONSTITUTIVE ACTIVITY OF NON-ENDOGENOUS GPCRS**

25 A variety of approaches are available for assessment of constitutive activity of the non-endogenous human GPCRs. The following are illustrative; those of ordinary skill in the art are credited with the ability to determine those techniques that are preferentially beneficial for the needs of the artisan.

**1. Membrane Binding Assays: [<sup>35</sup>S]GTPγS Assay**

30 When a G protein-coupled receptor is in its active state, either as a result of ligand binding or constitutive activation, the receptor couples to a G protein and stimulates the release of GDP and subsequent binding of GTP to the G protein. The alpha subunit of the G protein-receptor complex acts as a GTPase and slowly hydrolyzes

## 59.US1.PRO

## Provisional Application

the GTP to GDP, at which point the receptor normally is deactivated. Constitutively activated receptors continue to exchange GDP for GTP. The non-hydrolyzable GTP analog, [ $^{35}$ S]GTP $\gamma$ S, can be utilized to demonstrate enhanced binding of [ $^{35}$ S]GTP $\gamma$ S to membranes expressing constitutively activated receptors. The advantage of using [ $^{35}$ S]GTP $\gamma$ S binding to measure constitutive activation is that: (a) it is generically applicable to all G protein-coupled receptors; (b) it is proximal at the membrane surface making it less likely to pick-up molecules which affect the intracellular cascade.

The assay utilizes the ability of G protein coupled receptors to stimulate [ $^{35}$ S]GTP $\gamma$ S binding to membranes expressing the relevant receptors. The assay can, therefore, be used in the direct identification method to screen candidate compounds to known, orphan and constitutively activated G protein-coupled receptors. The assay is generic and has application to drug discovery at all G protein-coupled receptors.

The [ $^{35}$ S]GTP $\gamma$ S assay was incubated in 20 mM HEPES and between 1 and about 20mM MgCl<sub>2</sub> (this amount can be adjusted for optimization of results, although 20mM is preferred) pH 7.4, binding buffer with between about 0.3 and about 1.2 nM [ $^{35}$ S]GTP $\gamma$ S (this amount can be adjusted for optimization of results, although 1.2 is preferred ) and 12.5 to 75  $\mu$ g membrane protein (e.g. 293 cells expressing the Gs Fusion Protein; this amount can be adjusted for optimization) and 10  $\mu$ M GDP (this amount can be changed for optimization) for 1 hour. Wheatgerm agglutinin beads (25  $\mu$ l; Amersham) were then added and the mixture incubated for another 30 minutes at room temperature. The tubes were then centrifuged at 1500 x g for 5 minutes at room temperature and then counted in a scintillation counter.

## 2. Adenylyl Cyclase

A Flash Plate<sup>TM</sup> Adenylyl Cyclase kit (New England Nuclear, Cat. No. SMP004A) designed for cell-based assays can be modified for use with crude plasma membranes. The Flash Plate wells can contain a scintillant coating which also contains a specific antibody recognizing cAMP. The cAMP generated in the wells can be quantitated by a direct competition for binding of radioactive cAMP tracer to the cAMP antibody. The following serves as a brief protocol for the measurement of changes in cAMP levels in whole cells that express the receptors.

Transfected cells were harvested approximately twenty four hours after transient transfection. Media is carefully aspirated off and discarded. 10ml of PBS is gently added to each dish of cells followed by careful aspiration. 1ml of Sigma cell

## 59.US1.PRO

## Provisional Application

dissociation buffer and 3ml of PBS are added to each plate. Cells were pipetted off the plate and the cell suspension was collected into a 50ml conical centrifuge tube. Cells were then centrifuged at room temperature at 1,100 rpm for 5 min. The cell pellet was carefully re-suspended into an appropriate volume of PBS (about 3ml/plate). The cells were then counted using a hemocytometer and additional PBS was added to give the appropriate number of cells (with a final volume of about 50  $\mu$ l/well).

cAMP standards and Detection Buffer [comprising 1  $\mu$ Ci of tracer  $^{125}$ I-cAMP (50  $\mu$ l) to 11 ml Detection Buffer] was prepared and maintained in accordance with the manufacturer's instructions. Assay Buffer was prepared fresh for screening and contained 50 $\mu$ l of Stimulation Buffer, 3 $\mu$ l of test compound (12 $\mu$ M final assay concentration) and 50 $\mu$ l cells, Assay Buffer was stored on ice until utilized. The assay was initiated by addition of 50 $\mu$ l of cAMP standards to appropriate wells followed by addition of 50 $\mu$ l of PBSA to wells H-11 and H12. 50 $\mu$ l of Stimulation Buffer was added to all wells. DMSO (or selected candidate compounds) was added to appropriate wells using a pin tool capable of dispensing 3 $\mu$ l of compound solution, with a final assay concentration of 12 $\mu$ M test compound and 100 $\mu$ l total assay volume. The cells were then added to the wells and incubated for 60 min at room temperature. 100 $\mu$ l of Detection Mix containing tracer cAMP was then added to the wells. Plates were then incubated additional 2 hours followed by counting in a Wallac MicroBeta scintillation counter. Values of cAMP/well were then extrapolated from a standard cAMP curve which was contained within each assay plate.

### 3. Cell-Based cAMP for Gi Coupled Target GPCRs

TSHR is a Gs coupled GPCR that causes the accumulation of cAMP upon activation. TSHR will be constitutively activated by mutating amino acid residuc 623 (*i.e.*, changing an alanine residue to an isoleucine residue). A Gi coupled receptor is expected to inhibit adenylyl cyclase, and, therefore, decrease the level of cAMP production, which can make assessment of cAMP levels challenging. An effective technique for measuring the decrease in production of cAMP as an indication of constitutive activation of a Gi coupled receptor can be accomplished by co-transfecting, most preferably, non-endogenous, constitutively activated TSHR (TSHR-A623I) (or an endogenous, constitutively active Gs coupled receptor) as a "signal enhancer" with a Gi linked target GPCR to establish a baseline level of cAMP. Upon creating a non-endogenous version of the Gi coupled receptor, this non-endogenous version of the

## 59.US1.PRO

## Provisional Application

target GPCR is then co-transfected with the signal enhancer, and it is this material that can be used for screening. We will utilize such approach to effectively generate a signal when a cAMP assay is used; this approach is preferably used in the direct identification of candidate compounds against Gi coupled receptors. It is noted that for a Gi coupled GPCR, when this approach is used, an inverse agonist of the target GPCR will increase the cAMP signal and an agonist will decrease the cAMP signal.

On day one,  $2 \times 10^4$  293 cells/well will be plated out. On day two, two reaction tubes will be prepared (the proportions to follow for each tube are per plate): tube A will be prepared by mixing  $2 \mu\text{g}$  DNA of each receptor transfected into the mammalian cells, for a total of  $4 \mu\text{g}$  DNA (e.g., pCMV vector; pCMV vector with mutated THSR (TSHR-A623I); TSHR-A623I and GPCR, etc.) in 1.2ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B will be prepared by mixing  $120 \mu\text{l}$  lipofectamine (Gibco BRL) in 1.2ml serum free DMEM. Tubes A and B will then be admixed by inversions (several times), followed by incubation at room temperature for 30-45min. The admixture is referred to as the "transfection mixture". Plated 293 cells will be washed with 1XPBS, followed by addition of 10ml serum free DMEM. 2.4ml of the transfection mixture will then be added to the cells, followed by incubation for 4hrs at  $37^\circ\text{C}/5\% \text{CO}_2$ . The transfection mixture will then be removed by aspiration, followed by the addition of 25ml of DMEM/10% Fetal Bovine Serum. Cells will then be incubated at  $37^\circ\text{C}/5\% \text{CO}_2$ . After 24hr incubation, cells will then be harvested and utilized for analysis.

A Flash Plate™ Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) is designed for cell-based assays, however, can be modified for use with crude plasma membranes depending on the need of the skilled artisan. The Flash Plate wells will contain a scintillant coating which also contains a specific antibody recognizing cAMP. The cAMP generated in the wells can be quantitated by a direct competition for binding of radioactive cAMP tracer to the cAMP antibody. The following serves as a brief protocol for the measurement of changes in cAMP levels in whole cells that express the receptors.

Transfected cells will be harvested approximately twenty four hours after transient transfection. Media will be carefully aspirated off and discarded. 10ml of PBS will be gently added to each dish of cells followed by careful aspiration. 1ml of Sigma cell dissociation buffer and 3ml of PBS will be added to each plate. Cells will be pipetted off the plate and the cell suspension will be collected into a 50ml conical

## 59.US1.PRO

## Provisional Application

centrifuge tube. Cells will then be centrifuged at room temperature at 1,100 rpm for 5 min. The cell pellet will be carefully re-suspended into an appropriate volume of PBS (about 3ml/plate). The cells will then be counted using a hemocytometer and additional PBS is added to give the appropriate number of cells (with a final volume of about 50µl/well).

cAMP standards and Detection Buffer [comprising 1 µCi of tracer <sup>125</sup>I-cAMP (50 µl) to 11 ml Detection Buffer] will be prepared and maintained in accordance with the manufacturer's instructions. Assay Buffer should be prepared fresh for screening and contained 50µl of Stimulation Buffer, 3µl of test compound (12µM final assay concentration) and 50µl cells, Assay Buffer can be stored on ice until utilized. The assay can be initiated by addition of 50µl of cAMP standards to appropriate wells followed by addition of 50µl of PBSA to wells H-11 and H12. Fifty µl of Stimulation Buffer will be added to all wells. Selected compounds (e.g., TSH) will be added to appropriate wells using a pin tool capable of dispensing 3µl of compound solution, with a final assay concentration of 12µM test compound and 100µl total assay volume. The cells will then be added to the wells and incubated for 60 min at room temperature. 100µl of Detection Mix containing tracer cAMP will then be added to the wells. Plates were then incubated additional 2 hours followed by counting in a Wallac MicroBeta scintillation counter. Values of cAMP/well will then be extrapolated from a standard cAMP curve which is contained within each assay plate.

## Example 4

## TISSUE DISTRIBUTION OF THE DISCLOSED HUMAN GPCRS

## A. RT-PCR

RT-PCR was applied to confirm the expression and to determine the tissue distribution of several novel human GPCRs. Oligonucleotides utilized were GPCR-specific and the human multiple tissue cDNA panels (MTC, Clontech) as templates. Taq DNA polymerase (Stratagene) were utilized for the amplification in a 40µl reaction according to the manufacturer's instructions. 20µl of the reaction will be loaded on a 1.5% agarose gel to analyze the RT-PCR products. Table B below lists the receptors, the cycle conditions and the primers utilized.

TABLE B

59.US1.PRO

## Provisional Application

Receptor Identifier	Cycle Conditions Min ('), Sec (") Cycles 2-4 repeated 30 times	5' Primer (SEQ.ID.NO.)	3' Primer (SEQ.ID.NO.)	DNA Fragment	Tissue Expression
hRUP25	96° for 2' 96° for 30" 55°C for 1' 72° for 2' 72° for 10'	CTGATGGAC AACTATGTG AGGCGTTGG (5)	GCTGAAGCT GCTGCACAA ATTTGCACC (6)	297bp	Adipocyte, spleen, leukocyte, kidney, lung, testis

Diseases and disorders related to receptors located in these tissues or regions include, but are not limited to, cardiac disorders and diseases (e.g. thrombosis, myocardial infarction; atherosclerosis; cardiomyopathies); kidney disease/disorders (e.g., renal failure; renal tubular acidosis; renal glycosuria; nephrogenic type 2 diabetes insipidus; cystinuria; polycystic kidney disease); eosinophilia; leukocytosis; leukopenia; ovarian cancer; sexual dysfunction; polycystic ovarian syndrome; pancreatitis and pancreatic cancer; irritable bowel syndrome; colon cancer; Crohn's disease; ulcerative colitis; diverticulitis; Chronic Obstructive Pulmonary Disease (COPD); Cystic Fibrosis; pneumonia; pulmonary hypertension; tuberculosis and lung cancer; Parkinson's disease; movement disorders and ataxias; learning and memory disorders; eating disorders (e.g., anorexia; bulimia, etc.); obesity; cancers; thymoma; myasthenia gravis; circulatory disorders; prostate cancer; prostatitis; kidney disease/disorders(e.g., renal failure; renal tubular acidosis; renal glycosuria; nephrogenic type 2 diabetes insipidus; cystinuria; polycystic kidney disease); sensorimotor processing and arousal disorders; obsessive-compulsive disorders; testicular cancer; priapism; prostatitis; hernia; endocrine disorders; sexual dysfunction; allergies; depression; psychotic disorders; migraine; reflux; schizophrenia; ulcers; bronchospasm; epilepsy; prostatic hypertrophy; anxiety; rhinitis; angina; and glaucoma. Accordingly, the methods of the present invention may also be useful in the diagnosis and/or treatment of these and other diseases and disorders.

59.US1.PRO

## Provisional Application

**B. AFFYMETRIX GENECHIP® TECHNOLOGY**

Amino acid sequences were submitted to Affymetrix for the designing and manufacturing of microarray containing oligonucleotides to monitor the expression levels of G protein-coupled receptors (GPCRs) using their GeneChip® Technology.

Also present on the microarray were probes for characterized human brain tissues from Harvard Brain Bank or obtained from commercially available sources. RNA samples were amplified, labeled, hybridized to the microarray, and data analyzed according to manufacturer's instructions.

Adipose tissues were monitored for the level of gene expression of each of the GPCRs represented on the microarray. GPCRs were determined to be expressed if the expression index was greater than 100 (based upon and according to manufacturer's instructions). The data was analyzed and had indicated that classification of GPCRs with an expression index greater than 100 was reasonable because a number of known GPCRs had previously been reported to be expressed in neuronal tissues with an expression index greater than 100.

Using the GeneChip, we discovered **hRUP25** to have high levels of expression in adipocytes suggesting that, for example, that **hRUP25** may play a role in lipolysis (see, Goodman & Gilman's, The Pharmacological Basis of Therapeutics, 9<sup>th</sup> Edition, page 235 (1996). See Figure 1. Figure 1 is a plot representing the expression level of **hRUP25** in various tissues. Based upon this data, **hRUP25** is highly expressed by primary adipocytes.

This patent document discloses the identification of nicotinic acid as a ligand and agonist of human, mouse and rat **RUP25**. See, Examples *infra*.

**Example 5****Protocol: Direct Identification of Inverse Agonists and Agonists****A. [<sup>35</sup>S]GTPγS Assay**

Although we have utilized endogenous, constitutively active GPCRs for the direct identification of candidate compounds as, e.g., inverse agonists, for reasons that are not altogether understood, intra-assay variation can become exacerbated. In some embodiments, a GPCR Fusion Protein, as disclosed above, is also utilized with a non-endogenous, constitutively activated GPCR. When such a protein is used, intra-assay variation appears to be substantially stabilized, whereby an effective signal-to-noise ratio

59.US1.PRO

**Provisional Application**

is obtained. This has the beneficial result of allowing for a more robust identification of candidate compounds. Thus, in some embodiments it is preferred that for direct identification, a GPCR Fusion Protein be used and that when utilized, the following assay protocols be utilized.

5                   **1.     Membrane Preparation**

In some embodiments membranes comprising the constitutively active orphan GPCR/Fusion Protein of interest and for use in the direct identification of candidate compounds as inverse agonists or agonists are preferably prepared as follows:

**a.     Materials**

10                "Membrane Scrape Buffer" is comprised of 20mM HEPES and 10mM EDTA, pH 7.4; "Membrane Wash Buffer" is comprised of 20 mM HEPES and 0.1 mM EDTA, pH 7.4; "Binding Buffer" is comprised of 20mM HEPES, 100 mM NaCl, and 10 mM MgCl<sub>2</sub>, pH 7.4

**b.     Procedure**

15                All materials will be kept on ice throughout the procedure. Firstly, the media will be aspirated from a confluent monolayer of cells, followed by rinse with 10ml cold PBS, followed by aspiration. Thereafter, 5ml of Membrane Scrape Buffer will be added to scrape cells; this will be followed by transfer of cellular extract into 50ml centrifuge tubes (centrifuged at 20,000 rpm for 17 minutes at 4°C). Thereafter, the supernatant will  
20                be aspirated and the pellet will be resuspended in 30ml Membrane Wash Buffer followed by centrifuge at 20,000 rpm for 17 minutes at 4°C. The supernatant will then be aspirated and the pellet resuspended in Binding Buffer. This will then be homogenized using a Brinkman Polytron™ homogenizer (15-20 second bursts until the all material is in suspension). This is referred to herein as "Membrane Protein".

25                **2.     Bradford Protein Assay**

Following the homogenization, protein concentration of the membranes will be determined using the Bradford Protein Assay (protein can be diluted to about 1.5mg/ml, aliquoted and frozen (-80°C) for later use; when frozen, protocol for use will be as follows: on the day of the assay, frozen Membrane Protein is thawed at  
30                room temperature, followed by vortex and then homogenized with a Polytron at about 12 x 1,000 rpm for about 5-10 seconds; it was noted that for multiple preparations, the homogenizer should be thoroughly cleaned between homogenization of different preparations).

59.US1.PRO

# Provisional Application

## a. Materials

Binding Buffer (as per above); Bradford Dye Reagent; Bradford Protein Standard will be utilized, following manufacturer instructions (Biorad, cat. no. 500-0006).

## b. Procedure

Duplicate tubes will be prepared, one including the membrane, and one as a control "blank". Each contained 800µl Binding Buffer. Thereafter, 10µl of Bradford Protein Standard (1mg/ml) will be added to each tube, and 10µl of membrane Protein will then be added to just one tube (not the blank). Thereafter, 200µl of Bradford Dye Reagent will be added to each tube, followed by vortex of each. After five (5) minutes, the tubes will be re-vortexed and the material therein will be transferred to cuvettes. The cuvettes will then be read using a CECIL 3041 spectrophotometer, at wavelength 595.

## 3. Direct Identification Assay

### a. Materials

GDP Buffer consisted of 37.5 ml Binding Buffer and 2mg GDP (Sigma, cat. no. G-7127), followed by a series of dilutions in Binding Buffer to obtain 0.2 µM GDP (final concentration of GDP in each well was 0.1 µM GDP); each well comprising a candidate compound, has a final volume of 200µl consisting of 100µl GDP Buffer (final concentration, 0.1µM GDP), 50µl Membrane Protein in Binding Buffer, and 50µl [<sup>35</sup>S]GTPγS (0.6 nM) in Binding Buffer (2.5 µl [<sup>35</sup>S]GTPγS per 10ml Binding Buffer).

### b. Procedure

Candidate compounds will be preferably screened using a 96-well plate format (these can be frozen at -80°C). Membrane Protein (or membranes with expression vector excluding the GPCR Fusion Protein, as control), will be homogenized briefly until in suspension. Protein concentration will then be determined using the Bradford Protein Assay set forth above. Membrane Protein (and control) will then be diluted to 0.25mg/ml in Binding Buffer (final assay concentration, 12.5µg/well). Thereafter, 100 µl GDP Buffer was added to each well of a Wallac Scintistrip™ (Wallac). A 5ul pin-tool will then be used to transfer 5 µl of a candidate compound into such well (*i.e.*, 5µl in total assay volume of 200 µl is a 1:40 ratio such that the final screening concentration of the candidate compound is 10µM). Again, to avoid contamination, after each transfer step the pin tool should be rinsed in three reservoirs comprising water (1X), ethanol (1X)

## 59.UIS1.PRO

## Provisional Application

and water (2X) – excess liquid should be shaken from the tool after each rinse and dried with paper and kimwipes. Thereafter, 50  $\mu$ l of Membrane Protein will be added to each well (a control well comprising membranes without the GPCR Fusion Protein was also utilized), and pre-incubated for 5-10 minutes at room temperature. Thereafter, 50  $\mu$ l of [<sup>35</sup>S]GTP $\gamma$ S (0.6 nM) in Binding Buffer will be added to each well, followed by incubation on a shaker for 60 minutes at room temperature (again, in this example, plates were covered with foil). The assay will then be stopped by spinning of the plates at 4000 RPM for 15 minutes at 22°C. The plates will then be aspirated with an 8 channel manifold and sealed with plate covers. The plates will then be read on a Wallac 1450 using setting "Prot. #37" (as per manufacturer instructions).

**B. Cyclic AMP Assay**

Another assay approach to directly identified candidate compound was accomplished by utilizing a cyclase-based assay. In addition to direct identification, this assay approach can be utilized as an independent approach to provide confirmation of the results from the [<sup>35</sup>S]GTP $\gamma$ S approach as set forth above.

A modified Flash Plate™ Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) was preferably utilized for direct identification of candidate compounds as inverse agonists and agonists to constitutively activated orphan GPCRs in accordance with the following protocol.

Transfected cells were harvested approximately three days after transfection. Membranes were prepared by homogenization of suspended cells in buffer containing 20mM HEPES, pH 7.4 and 10mM MgCl<sub>2</sub>. Homogenization was performed on ice using a Brinkman Polytron™ for approximately 10 seconds. The resulting homogenate is centrifuged at 49,000 X g for 15 minutes at 4°C. The resulting pellet was then resuspended in buffer containing 20mM HEPES, pH 7.4 and 0.1 mM EDTA, homogenized for 10 seconds, followed by centrifugation at 49,000 x g for 15 minutes at 4°C. The resulting pellet was then stored at -80°C until utilized. On the day of direct identification screening, the membrane pellet was slowly thawed at room temperature, resuspended in buffer containing 20mM HEPES, pH 7.4 and 10mM MgCl<sub>2</sub>, to yield a final protein concentration of 0.60mg/ml (the resuspended membranes are placed on ice until use).

cAMP standards and Detection Buffer [comprising 2  $\mu$ Ci of tracer <sup>125</sup>I-cAMP (100  $\mu$ l) to 11 ml Detection Buffer] were prepared and maintained in accordance with

## 59.US1.PRO

## Provisional Application

the manufacturer's instructions. Assay Buffer was prepared fresh for screening and contained 20mM HEPES, pH 7.4, 10mM MgCl<sub>2</sub>, 20mM phosphocreatine (Sigma), 0.1 units/ml creatine phosphokinase (Sigma), 50 μM GTP (Sigma), and 0.2 mM ATP (Sigma); Assay Buffer was then stored on ice until utilized.

5       Candidate compounds identified as per above (if frozen, thawed at room temperature) were added, preferably, to 96-well plate wells (3μl/well; 12μM final assay concentration), together with 40 μl Membrane Protein (30μg/well) and 50μl of Assay Buffer. This admixture was then incubated for 30 minutes at room temperature, with gentle shaking.

10       Following the incubation, 100μl of Detection Buffer was added to each well, followed by incubation for 2-24 hours. Plates were then counted in a Wallac MicroBeta™ plate reader using "Prot. #31" (as per manufacturer instructions).

## Example 6

## 15       MELANOPHORE TECHNOLOGY

Melanophores are skin cells found in lower vertebrates. They contain pigmented organelles termed melanosomes. Melanophores are able to redistribute these melanosomes along a microtubule network upon G-protein coupled receptor (GPCR) activation. The result of this pigment movement is an apparent lightening or darkening of the cells. In melanophores, the decreased levels of intracellular cAMP that result from activation of a Gi-coupled receptor cause melanosomes to migrate to the center of the cell, resulting in a dramatic lightening in color. If cAMP levels are then raised, following activation of a Gs-coupled receptor, the melanosomes are re-dispersed and the cells appear dark again. The increased levels of diacylglycerol that result from activation of Gq-coupled receptors can also induce this re-dispersion. In addition, the technology is also suited to the study of certain receptor tyrosine kinases. The response of the melanophores takes place within minutes of receptor activation and results in a simple, robust color change. The response can be easily detected using a conventional absorbance microplate reader or a modest video imaging system. Unlike other skin cells, the melanophores derive from the neural crest and appear to express a full complement of signaling proteins. In particular, the cells express an extremely wide range of G-proteins and so are able to functionally express almost all GPCRs.

Melanophores can be utilized to identify compounds, including natural ligands, against GPCRs. This method can be conducted by introducing test cells of a pigment

59.US1.PRO

## Provisional Application

cell line capable of dispersing or aggregating their pigment in response to a specific stimulus and expressing an exogenous clone coding for the GPCR. A stimulant, e.g., melatonin, sets an initial state of pigment disposition wherein the pigment is aggregated within the test cells if activation of the GPCR induces pigment dispersion. However, stimulating the cell with a stimulant to set an initial state of pigment disposition wherein the pigment is dispersed if activation of the GPCR induces pigment aggregation. The test cells are then contacted with chemical compounds, and it is determined whether the pigment disposition in the cells changed from the initial state of pigment disposition. Dispersion of pigments cells due to the candidate compound, including but not limited to a ligand, coupling to the GPCR will appear dark on a petri dish, while aggregation of pigments cells will appear light.

Materials and methods will be followed according to the disclosure of U.S. Patent Number 5,462,856 and U.S. Patent Number 6,051,386. These patent disclosures are hereby incorporated by reference in their entirety.

Melanophores were transfected by electroporation with plasmids coding for the GPCRs, for example **hRUP25**. Pre-screening of the GPCRs in melanophores was performed in the absence of nicotinic acid following the protocol below to determine the G protein coupling. This pre-screen evidenced that **hRUP25** (Figure 2) is strongly Gi-coupled.

The cells were plated in 96-well plates (one receptor per plate). 48 hours post-transfection, half of the cells on each plate were treated with 10nM melatonin. Melatonin activates an endogenous Gi-coupled receptor in the melanophores and causes them to aggregate their pigment. The remaining half of the cells were transferred to serum-free medium 0.7X L-15 (Gibco). After one hour, the cells in serum-free media remained in a pigment-dispersed state while the melatonin-treated cells were in a pigment-aggregated state. At this point, the cells were treated with a dose response of nicotinic acid (Sigma). If the plated GPCRs bound to nicotinic acid, the melanophores would be expected to undergo a color change in response to the compound. If the receptor were either a Gs or Gq coupled receptor, then the melatonin-aggregated melanophores would undergo pigment dispersion. In contrast, if the receptor was a Gi-coupled receptor, then the pigment-dispersed cells would be expected to undergo a dose-dependent pigment aggregation.

Melanophores transfected with **hRUP25** were treated with nicotinic acid. Upon this treatment, the cells underwent pigment aggregation in a dose-dependent manner.

59.US1.PRO

## Provisional Application

**hRUP25**-expressing cells that were pre-aggregated with melatonin did not disperse upon nicotinic acid treatment, which is consistent with the receptor being a Gi-coupled receptor. See, Figure 3 and *infra*.

To confirm and extend these results, melanophores were transfected with a range of **hRUP25** DNA from 0 to 10 $\mu$ g. As controls, melanophores were also transfected with 10 $\mu$ g of  $\alpha_{2A}$  Adrenergic receptor (a known Gi-coupled receptor) and salmon sperm DNA (Gibco), as a mock transfection. On day 3, the cells were again incubated for 1 hour in serum-free L-15 medium (Gibco) and remained in a pigment-dispersed state. The cells were then treated with a dose response of nicotinic acid. See, Figure 3A. Figure 3A depicts the aggregation response of nicotinic acid at melanophores transfected with various ranges of **hRUP25**. At 10 $\mu$ g of **hRUP25**, the EC<sub>50</sub> for nicotinic acid is about 54nM. Stated differently, at very low concentrations, nicotinic acid evidences binding to **hRUP25**.

Reference is now made to Figure 3B. In Figure 3B, both the mock transfected and  $\alpha_{2A}$  transfected cells did not respond to nicotinic acid. This data evidences that nicotinic acid binds specifically to the Gi-coupled receptor **hRUP25**.

The data show that the greater the amount of **hRUP25** plasmid DNA transfected, the greater the magnitude of the observed aggregation response. Collectively these data indicate that **hRUP25**: 1) is a Gi-coupled receptor that 2) binds to nicotinic acid.

As set forth herein, nicotinic acid is a ligand for, and agonist of, human, mouse and rat **RUP25**. It is further shown that human, mouse and rat **RUP25** are Gi-coupled. Additionally, human, mouse, and rat **RUP25** can be used in methods described herein to identify antagonists, agonists, inverse agonists, partial agonists, allosteric enhancers, and negative allosteric modulators. As discussed *supra*, methods of modifying nicotinic acid receptor activity in adipocytes using a modulator of the receptor are set forth. Preferably, the modulator is an agonist.

## Example 7

**NICOTINIC ACID INDUCED-INOSITOL PHOSPHATES ACCUMULATION IN 293 CELLS CO-EXPRESSING hRUP AND GqAGi**

## 59.US1.PRO

## Provisional Application

Figure 4 illustrates the nicotinic acid induced-inositol phosphates (IPs) accumulation in HEK293 cells co-expressing **hRUP25** and the chimeric G $\alpha$ q-subunit in which the last five amino acids have been replaced with the corresponding amino acids of G $\alpha$ i (Gq $\Delta$ Gi). This construct has been shown to convert the signaling of a Gi-coupled receptor to the Gq pathway (i.e. accumulation of inositol phosphates) in response to receptor activation. Cells transfected with Gq $\Delta$ Gi plus either empty plasmid or the constitutively activated  $\alpha_{2A}$ AR ( $\alpha_{2A}$ K) served as controls for the IP assay which are non-responsive to nicotinic acid.

## Example 8

**NICOTINIC ACID AND NICOTINE INDUCED-INHIBITION OF FORSKOLIN STIMULATED cAMP ACCUMULATION IN hRUP25-CHO CELL STABLE LINE #46**

Figure 5A is a set of immunofluorescent photomicrographs illustrating the expression of hemagglutinin(HA)-tagged **hRUP25** in a stably transfected line of CHO cells (top; clone #46). No significant labeling is detected in mock stably-transfected CHO cells (Mock). The lower panels identify the nuclear (DAPI) staining of cells in the same field.

Figure 5B illustrates nicotinic acid and nicotine induced-inhibition of forskolin stimulated cAMP accumulation in **hRUP25-CHO** cell stable line #46 (described in preceding paragraph). The EC<sub>50</sub> for nicotinic acid is 23.6nM and that for nicotine is 9.8 $\mu$ M.

## Example 9

**hRUP25 AND mRUP25 INHIBIT TSHR INDUCED-cAMP ACCUMULATION FOLLOWING ACTIVATION BY NICOTINIC ACID**

Figure 6 indicates that, in response to nicotinic acid, both **hRUP25** and the mouse ortholog **mRUP25** can inhibit TSHR stimulated cAMP production (in the presence and absence of TSH).

## Example 10

59.US1.PRO

## Provisional Application

**hRUP25 AND mRUP25 BIND TO NICOTINIC ACID SPECIFICALLY AND WITH HIGH AFFINITY**

Figure 7 shows the saturation binding curves of [ $^3$ H]nicotinic acid ([ $^3$ H]NA) to membranes prepared from HEK293 cells transiently expressing either **hRUP25** or **mRUP25**. Note the significant binding of [ $^3$ H]NA relative to either that found in membranes derived from mock transfected cells or in the presence of an excess of non-labeled nicotinic acid (200 $\mu$ M).

Radioligand binding was done as follows. Media was removed from cells grown in culture [either stably or transiently transfected with negative control (empty plasmid) or with the individual receptors **hRUP25**, **mRUP25**, **rRUP25** and cells were scraped and homogenized in buffer containing 15mM HEPES, 5mM EDTA, 5mM EGTA, plus protease inhibitors (leupeptin, PMSF and pepstatin). Membranes were harvested following centrifugation at 30,000 X g, 4°C for 30min. Membranes were then resuspended and re-homogenized in CHAPS binding buffer (50mM Tris-HCl and 0.02% CHAPS, pH 7.4). Aliquots were taken for protein analysis *via* the Bradford protein assay and normalized such that each binding reaction contained the same amount of membrane protein (25-50 $\mu$ g). 50nM [ $^3$ H]nicotinic acid was added to each sample and either buffer (for total samples) or a desired amount of non-labeled compound (at the same volumes and in the same diluent) was added and the reactions were left at room temperature gently shaking for 1hr. Free ligand was separated from bound ligand *via* rapid filtration onto a filter. Appropriate scintillant was added to each sample and counted in an appropriate scintillation counter. Data was analyzed using Excel and PrismGraph. In some cases radioligand binding was performed using a scintillation proximity assay (SPA) in which case the samples did not require filtration or the addition of scintillant.

**Example 11**

**THE RANK ORDER OF POTENCY OF COMPOUNDS ON hRUP25 CLOSELY MATCHES THAT OF THE PHARMACOLOGICALLY DEFINED NICOTINIC ACID RECEPTOR**

Figure 8 is a table comparing the rank order of potency of various compounds on **hRUP25** and the pharmacologically defined nicotinic acid receptor. The potencies at

59.US1.PRO

## Provisional Application

hRUP25 derived both by a functional analysis measuring the inhibition of forskolin induced cAMP production and competitive radioligand binding assays, closely match the order of potencies of the pharmacologically defined nicotinic acid receptor.

5      **Example 12**

**NICOTINIC ACID AND RELATED COMPOUNDS INHIBIT ISOPROTERENOL INDUCED LIPOLYSIS IN RAT EPIDIDMAL FAT DERIVED ADIPOCYTES**

10      Figure 9A depicts nicotinic acid and related compounds inhibiting isoproterenol induced lipolysis in rat epididmal fat derived adipocytes at a concentration of 10 $\mu$ M. P-3-T represents 3-tetrazole-5-pyridine.

Figure 9B illustrates a nicotinic acid dose-dependent inhibition of isoproterenol induced-lipolysis in rat epididmal fat derived adipocytes. Note the rightward shift in the dose-response curves with increasing concentrations of nicotinic acid.

15      Lipolysis assays were done following the isolation of adipocytes from rat or human. The source of fat from rats was the epididymal fat and from humans was either subcutaneous or omental. Cells were isolated following collagenase digestion and floatation. An elevation of intracellular cAMP levels and concomitant activation of lipolysis *via* hormone sensitive lipase was accomplished using isoproterenol, forskolin,  
20      3-isobutyl-1-methyl-xanthine (IBMX) or a combination thereof at concentrations and times determined empirically and depending on the source of tissue. Lipolysis was allowed to continue for the desired time in the presence or absence of drug (*e.g.* nicotinic acid, P-3-T, etc). Data was analyzed using Excel and PrismGraph.

25      **Example 13**

**DOSE-DEPENDENT INHIBITION OF ISOPROTERENOL INDUCED-LIPOLYSIS IN HUMAN, SUBCUTANEOUS-DERIVED, PRIMARY ADIPOCYTES VIA NICOTINIC ACID AND P-3-T**

30      Figure 10 illustrates the ability of both nicotinic acid and the related compound P-3-T (3-tetrazole-5-pyridine) to inhibit isoproterenol induced lipolysis in adipocyte primary cultures derived from human subcutaneous fat in a dose-dependant manner. The EC<sub>50</sub> value for nicotinic acid and P-3-T were 716nM and 218nM respectively.

59.US1.PRO

Provisional Application

## Example 14

SUMMARY: hRUP25, mRUP25 AND rRUP25.

TABLE C

Disclosed Nicotinic Acid Receptor Sub-Family GPCRs	Expression by Adipocytes or Adipose	Gi-Coupled (Lowers the Level of Intracellular cAMP)	Shown to Inhibit Intracellular Lipolysis	Agonist
hRUP25	yes	yes	yes	nicotinic acid; nicotine; <i>see</i> Figure 8
mRUP25	yes	yes	yes	nicotinic acid
rRUP25	yes	yes	yes	nicotinic acid

## Example 15

## RODENT DIABETES MODELS

Rodent models of type 2 diabetes associated with obesity and insulin resistance have been developed. Genetic models such as db/db and ob/ob [*see* Diabetes (1982) 31:1-6] in mice and fa/fa in zucker rats have been developed for understanding the pathophysiology of disease and for testing candidate therapeutic compounds [Diabetes (1983) 32:830-838; Annu Rep Sankyo Res Lab (1994) 46:1-57]. The homozygous animals, C57 BL/KsJ-db/db mice developed by Jackson Laboratory are obese, hyperglycemic, hyperinsulinemic and insulin resistant [J Clin Invest (1990) 85:962-967], whereas heterozygotes are lean and normoglycemic. In the db/db model, mice progressively develop insulinopenia with age, a feature commonly observed in late stages of human type 2 diabetes when sugar levels are insufficiently controlled. Since this model resembles that of human type 2 diabetes, the compounds of the present invention are tested for activities including, but not limited to, lowering of plasma glucose and triglycerides. Zucker (fa/fa) rats are severely obese, hyperinsulinemic, and insulin resistant [Coleman, Diabetes (1982) 31:1; E Shafir in Diabetes Mellitus, H Rifkin and D Porte, Jr, Eds [Elsevier Science Publishing Co, New York, ed. 4, (1990), pp. 299-340]], and the fa/fa mutation can be the rat equivalent of the murine db mutation

59.US1.PRO

## Provisional Application

[Friedman et al, Cell (1992) 69:217-220; Truett et al, Proc Natl Acad Sci USA (1991) 88:7806]. Tubby (tub/tub) mice are characterized by obesity, moderate insulin resistance and hyperinsulinemia without significant hyperglycemia [Coleman et al, Heredity (1990) 81:424].

5 The present invention encompasses the use of compounds of the invention for reducing the insulin resistance and hyperglycemia in any or all of the above rodent diabetes models, in humans with type 2 diabetes or other preferred metabolic-related disorders or disorders of lipid metabolism described previously, or in models based on other mammals. Plasma glucose and insulin levels will be tested, as well as other factors including, but not limited to, plasma free fatty acids and triglycerides.

10 In Vivo Assay for Anti-Hyperglycemic Activity of Compounds of the Invention

Genetically altered obese diabetic mice (db/db) (male, 7-9 weeks old) are housed (7-9 mice/cage) under standard laboratory conditions at 22°C and 50% relative humidity, and maintained on a diet of Purina rodent chow and water *ad libitum*. Prior to treatment, blood is collected from the tail vein of each animal and blood glucose concentrations are determined using One Touch Basic Glucose Monitor System (Lifescan). Mice that have plasma glucose levels between 250 to 500 mg/dl are used. Each treatment group consists of seven mice that are distributed so that the mean glucose levels are equivalent in each group at the start of the study. The db/db mice are dosed by micro-osmotic pumps, inserted using isoflurane anesthesia, to provide compounds of the invention, saline, or an irrelevant compound to the mice subcutaneously (s.c.). Blood is sampled from the tail vein at intervals thereafter and analyzed for blood glucose concentrations. Significant differences between groups (comparing compounds of the invention to saline-treated) are evaluated using Student t-test.

25 **Example 16**

**MOUSE ATHEROSCLEROSIS MODEL**

Adiponectin-deficient mice generated through knocking out the adiponectin gene have been shown to be predisposed to atherosclerosis and to be insulin resistant. The mice are also a suitable model for ischemic heart disease [Matsuda, M et al. J Biol Chem (2002) July, and references cited therein, the disclosures of which are incorporated herein by reference in their entirety].

Adiponectin knockout mice are housed (7-9 mice/cage) under standard laboratory conditions at 22°C and 50% relative humidity. The mice are dosed by micro-

59.US1.PRO

## Provisional Application

osmotic pumps, inserted using isoflurane anesthesia, to provide compounds of the invention, saline, or an irrelevant compound to the mice subcutaneously (s.c.). Neointimal thickening and ischemic heart disease are determined for different groups of mice sacrificed at different time intervals. Significant differences between groups (comparing compounds of the invention to saline-treated) are evaluated using Student t-test.

## Example 17

*In Vivo* Animal Model For Dyslipidemia and Atherosclerosis

The utility of the compound of the present invention as a medical agent in the prophylaxis and treatment of a high total cholesterol/HDL-cholesterol ratio and conditions relating thereto is demonstrated by the activity of the compound in lowering the ratio of total cholesterol to HDL-cholesterol, in elevating HDL-cholesterol, or in protection from atherosclerosis in an *in vivo* pig model. Pigs are used as an animal model because they reflect human physiology, especially lipid metabolism, more closely than most other animal models. An illustrative *in vivo* pig model not intended to be limiting is presented here.

Yorkshire albino pigs (body weight  $25.5 \pm 4$  kg) are fed a saturated fatty acid rich and cholesterol rich (SFA-CHO) diet during 50 days (1 kg chow  $35 \text{ kg}^{-1}$  pig weight), composed of standard chow supplemented with 2% cholesterol and 20% beef tallow [Royo T et al., *European Journal of Clinical Investigation* (2000) 30:843-52; which disclosure is hereby incorporated by reference in its entirety]. Saturated to unsaturated fatty acid ratio is modified from 0.6 in normal pig chow to 1.12 in the SFA-CHO diet. Animals are divided into two groups, one group ( $n = 8$ ) fed with the SFA-CHO diet and treated with placebo and one group ( $n = 8$ ) fed with the SFA-CHO diet and treated with the compound ( $3.0 \text{ mg kg}^{-1}$ ). Control animals are fed a standard chow for a period of 50 days. Blood samples are collected at baseline (2 days after the reception of the animals), and 50 days after the initiation of the diet. Blood lipids are analyzed. The animals are sacrificed and necropsied.

Alternatively, the foregoing analysis comprises a plurality of groups each treated with a different dose of the compound. Preferred said doses are selected from the group consisting of:  $0.1 \text{ mg kg}^{-1}$ ,  $0.3 \text{ mg kg}^{-1}$ ,  $1.0 \text{ mg kg}^{-1}$ ,  $3.0 \text{ mg kg}^{-1}$ ,  $10 \text{ mg kg}^{-1}$ ,  $30 \text{ mg kg}^{-1}$  and  $100 \text{ mg kg}^{-1}$ . Alternatively, the foregoing analysis is carried out at a plurality of

59.US1.PRO

Provisional Application

timepoints. Preferred said timepoints are selected from the group consisting of 10 weeks, 20 weeks, 30 weeks, 40 weeks, and 50 weeks.

HDL-Cholesterol

5 Blood is collected in trisodium citrate (3.8%, 1:10). Plasma is obtained after centrifugation (1200 g 15 min) and immediately processed. Total cholesterol, HDL-cholesterol, and LDL-cholesterol are measured using the automatic analyzer Kodak Ektachem DT System (Eastman Kodak Company, Rochester, NY, USA). Samples with value parameters above the range are diluted with the solution supplied by the  
10 manufacturer and then re-analyzed. The total cholesterol/HDL-cholesterol ratio is determined. Comparison is made of the level of HDL-cholesterol between groups. Comparison is made of the total cholesterol/HDL-cholesterol ratio between groups.

Elevation of HDL-cholesterol or reduction of the total cholesterol/HDL-cholesterol ratio on administration of the compound is taken as indicative of the  
15 compound having the aforesaid utility.

Atherosclerosis

The thoracic and abdominal aortas are removed intact, opened longitudinally along the ventral surface, and fixed in neutral-buffered formalin after excision of  
20 samples from standard sites in the thoracic and abdominal aorta for histological examination and lipid composition and synthesis studies. After fixation, the whole aortas are stained with Sudan IV and pinned out flat, and digital images are obtained with a TV camera connected to a computerized image analysis system (Image Pro Plus; Media Cybernetics, Silver Spring, MD) to determine the percentage of aortic surface  
25 involved with atherosclerotic lesions [Gerrity RG et al, *Diabetes* (2001) 50:1654-65; Cornhill JF et al, *Arteriosclerosis, Thrombosis, and Vascular Biology* (1985) 5:415-26; which disclosures are hereby incorporated by reference in their entirety]. Comparison is made between groups of the percentage of aortic surface involved with atherosclerotic lesions.

30 Reduction of the percentage of aortic surface involved with atherosclerotic lesions on administration of the compound is taken as indicative of the compound having the aforesaid utility.

## 59.US1.PRO

## Provisional Application

## Example 18

*In Vitro* Biological Activity

A modified Flash Plate™ Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) is used for direct identification of candidate compounds as agonists to hRUP25 in accordance with the following protocol:

Stably transfected CHO cells (clone 46) were harvested from flasks *via* non-enzymatic means. The cells were washed in PBS and resuspended in the manufacturer's Assay Buffer. Live cells were counted using a hemacytometer and Trypan blue exclusion, and the cell concentration was adjusted to  $2 \times 10^6$  cells/ml. cAMP standards and Detection Buffer (comprising 2  $\mu$ Ci of tracer [ $^{125}$ I]-cAMP (100  $\mu$ l) to 11 ml Detection Buffer) were prepared and maintained in accordance with the manufacturer's instructions. Candidate compounds identified as per above (if frozen, thawed at room temperature) were added to their respective wells (preferably wells of a 96-well plate) at increasing concentrations (3  $\mu$ l/well; 12  $\mu$ M final assay concentration). To these wells, 100,000 cells in 50  $\mu$ l of Assay Buffer were added and the mixture was then incubated for 30 minutes at room temperature, with gentle shaking. Following the incubation, 100  $\mu$ l of Detection Buffer was added to each well, followed by incubation for 2-24 hours. Plates were counted in a Wallac MicroBeta™ plate reader using "Prot. #31" (as per manufacturer instructions).

The biological activities for several representative compounds using the above mentioned assay are shown in the table below:

Compound No.	RUP25 (EC <sub>50</sub> ) ( $\mu$ M)
48	4.3
63	7.9

The majority of the compounds of the Examples showed activities of at least about 60  $\mu$ M.

## Example 19

## General Synthesis of compounds of Formula (I) - Pyrazole Formation:

To a solution of NaOEt in EtOH (either prepared by the addition of Na or commercially available NaOEt; 17.4 mmol; EtOH 20 mL), is added a ketone (15 mmol) and diethyl oxalate (2.2 g, 15 mmol) at room temperature. The reaction is heated

## 59.US1.PRO

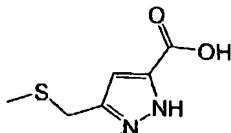
## Provisional Application

to 75 °C and maintained at the same temperature for two hours. The reaction is cooled to room temperature and treated with a solution of  $\text{NH}_2\text{NH}_2 \cdot \text{HCl}$  (1.57 g 15 mmol) in  $\text{H}_2\text{O}$  (3 mL). The subsequent reaction mixture is allowed to stir at 75 °C for two hours. The reaction is cooled to room temperature and concentrated under vacuum. The resulting residue is dissolved in 2.5M NaOH (10 mL) and heated to 95 °C. After stirring for two hours, the reaction is cooled to room temperature and washed with ether (5 mL). The aqueous layer is neutralized (pH = 6.5) with 2M HCl at 0 °C. The reaction is stirred at 0 °C for one hour and the product is filtered. The solid is washed with  $\text{H}_2\text{O}$  (10 mL) and dried under vacuum to afford the desired compound. The product if not a solid can be purified via methods known in the art, for example, by column chromatography or HPLC.

Utilizing the above procedure with 2-hexanone (1.5 g, 15 mmol) gave the desired product, as the carboxylic acid after hydrolysis, 1.69 g (67% yield, unoptimized).

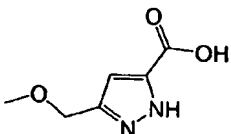
**Representative compounds of the present invention:**

Compound 48: 5-Methylsulfanylmethyl-2H-pyrazole-3-carboxylic acid.



Compound 48 was prepared using a similar method as described above; LCMS: 517.4 (3M+H)<sup>+</sup>, 345.2 (2M+H)<sup>+</sup>, 173.1 (MH)<sup>+</sup>, 154.9, 125.1 and 107.0. NMR (400MHz, CD<sub>3</sub>OD, ppm): 6.62 (1H, s), 3.63 (2H, s) and 3.3 (3H, s).

Compound 63: 5-Methoxymethyl-2H-pyrazole-3-carboxylic acid.

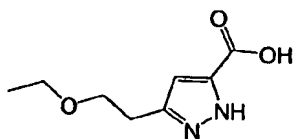


Compound 63 was prepared using a similar method as described above; LCMS: 469.3 (3M+H)<sup>+</sup>, 313.2 (2M+H)<sup>+</sup>, 157.0 (MH)<sup>+</sup>, 139.0, 125.1 and 107.1. NMR (400MHz, DMSO-d<sub>6</sub>, ppm): 13.2 (1H, br. s), 6.75 (1H, s), 4.45 (2H, s) and 3.3 (3H, s).

Compound 64: 5-(2-Ethoxy-ethyl)-2H-pyrazole-3-carboxylic acid.

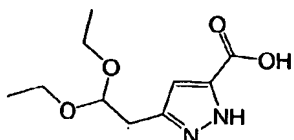
59.US1.PRO

Provisional Application



Compound 64 was prepared using a similar method as described above; LCMS:  
 553.5 (3M+H)<sup>+</sup>, 369.2 (2M+H)<sup>+</sup>, 185.0 (MH)<sup>+</sup>, 167.1 and 121.2. NMR (400MHz,  
 DMSOd6, ppm): 13.1 (1H, br. s), 6.6 (1H, s), 3.68 (2H, t, *J*= 8Hz), 3.5 (2H, q, *J*= 8Hz),  
 2.88 (2H, t, *J*= 8Hz) and 1.15 (3H, t, *J*= 8Hz).

Compound 65: 5-(2,2-Diethoxy-ethyl)-2H-pyrazole-3-carboxylic acid.



Compound 65 was prepared using a similar method as described above; LCMS:  
 685.5 (3M+H)<sup>+</sup>, 457.2 (2M+H)<sup>+</sup>, 229.2 (MH)<sup>+</sup>, 183.0 and 137.0. NMR (400MHz,  
 DMSOd6, ppm): 13 (1H, br. s), 6.55 (1H, s), 4.7 (1H, t, *J*= 4Hz), 3.6 and 3.58 (2H, qq,  
*J*= 8Hz), 3.44 and 3.42 (2H, qq, *J*= 8Hz), 2.86 (2H, d, *J*= 8Hz) and 1.05 (6H, t, *J*= 8Hz).

### Example 20

#### General Synthesis of compounds of Formula (I) - Reductive amination:

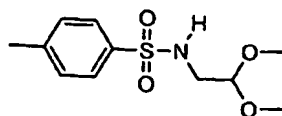
To a 20 mL vial with stirring bar is added 5-formyl-1H-pyrazole-3-carboxylic acid ethyl ester (1.19 mmol) and 1,2-dichloroethane (3 mL). An amine (1.19 mmol) is added, followed by sodium triacetoxyborohydride (2.37 mmol). The vial is capped with a septum flushed with N<sub>2</sub>, and stirred overnight at room temperature. The mixture is added to NaHCO<sub>3</sub> (10 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL). The combined organic extracts are dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product is purified using preparatory-HPLC or other purification method known in the art to provide compounds of Formula (I), where R<sub>2</sub> is not H. The purified product can be hydrolysis, for example, using a manner as described above in Example 19, to give compounds of Formula (I), where R<sub>2</sub> is H.

The intermediate 5-formyl-1H-pyrazole-3-carboxylic acid ethyl ester was prepared in the following manner:

Step A: Synthesis of N-(2,2-Dimethoxy-ethyl)-4-methyl-benzenesulfonamide.

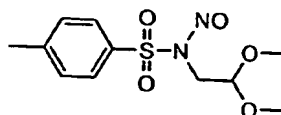
59.US1.PRO

## Provisional Application



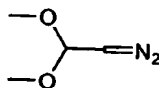
To 350 mL of dry Et<sub>2</sub>O was added p-toluenesulfonyl chloride (73.4 g, 385 mmol). Without cooling, a mixture of 2,2-dimethoxy-ethylamine (36.8 g, 350 mmol) and triethylamine (53.7 mL, 385 mmol) was added dropwise to the first solution at a rate sufficient to give a gentle reflux. After stirring 15 h, the reaction was washed with NaHCO<sub>3</sub>, and the organic layer was separated, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated providing 95.0 g (95%) of N-(2,2-dimethoxy-ethyl)-4-methyl-benzenesulfonamide.

Step B: Synthesis of N-(2,2-dimethoxyethyl)-N-nitroso-4-tosylamide.



Without further purification, N-(2,2-dimethoxyethyl)-4-methyl-benzenesulfonamide (95 g, 366 mmol) was taken up in dry Et<sub>2</sub>O (350 mL) and treated with HOAc (140 mL), and Ac<sub>2</sub>O (140 mL), cooled to 0 °C and stirred. In one portion, NaNO<sub>2</sub> (48.3 g, 700 mmol) was added, and the reaction was stirred 2 h at 0 °C, and then 15 h at rt. Saturated NaHCO<sub>3</sub> (350 mL) was added, followed by solid NaHCO<sub>3</sub> until CO<sub>2</sub> bubbling stopped. The organic layer was separated, and the aqueous portion was extracted with Et<sub>2</sub>O (4 x 100 mL). The combined organic layers were washed with NaHCO<sub>3</sub> (2 x 50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated providing 36.4 g (34%) of N-(2,2-dimethoxyethyl)-N-nitroso-4-tosylamide.

Step C: Synthesis of 2-Diazo-1,1-dimethoxy-ethane.

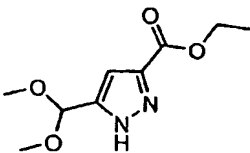


To a mixture of MeOH (140 mL), H<sub>2</sub>O (70 mL), and Et<sub>2</sub>O (28 mL) was added KOH (21.1 g, 377 mmol). After the KOH dissolved, the mixture was stirred vigorously at 0 °C. Portionwise, N-(2,2-dimethoxyethyl)-N-nitroso-4-tosylamide (36.4 g, 126 mmol) was added over 15 min., and stirred 2 h at 0 °C. Ether (70 mL) and 2 M KOH (70 mL) were added, and the reaction was stirred 2 h at 0 °C. Further 2 M KOH (70 mL) was added to dissolve any solid, and the Et<sub>2</sub>O layer was separated. The aqueous portion was extracted with Et<sub>2</sub>O until no more yellow color was seen in the organic phase. The combined organic extracts were washed with 2 M KOH (70 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and filtered to provide a solution of 2-diazo-1,1-dimethoxy-ethane in ether.

59.US1.PRO

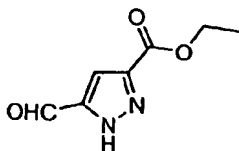
## Provisional Application

Step D: Synthesis of 5-Dimethoxymethyl-1H-pyrazole-3-carboxylic acid ethyl ester.



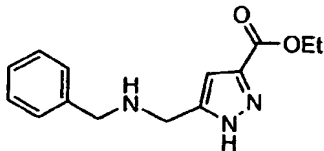
The ether solution of 2-diazo-1,1-dimethoxy-ethane in ether from the previous step was cooled to about -20 °C and treated with propynoic acid ethyl ester (12.8 mL, 126 mmol). The reaction was stirred for 2 h at 0 °C, quenched with AcOH (1.8 mL), and concentrated to afford ~27.4 g of 5-dimethoxymethyl-1H-pyrazole-3-carboxylic acid ethyl ester.

Step E: Synthesis of 5-formyl-1H-pyrazole-3-carboxylic acid ethyl ester.



Amberlyst-15 (19 g) was added to a solution of crude 5-dimethoxymethyl-1H-pyrazole-3-carboxylic acid ethyl ester dissolved in acetone (142 mL) and H<sub>2</sub>O (66 mL). After stirring 2 h at rt, the Amberlyst was filtered from the solution, and the filtrate was extracted with EtOAc (2 x 100 mL) and concentrated. The concentrate was suspended in benzene (30 mL) and hexane (120 mL), and stirred 2 h at rt. The precipitate was filtered, washed with 9:1 hexanes/benzene, and air-dried providing 5.29 g [25% from N-(2,2-dimethoxyethyl)-N-nitroso-4-tosylamide] of 5-dimethoxymethyl-1H-pyrazole-3-carboxylic acid ethyl ester: LC-MS *m/z* 167 (M-1); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.97 (bs, 1 H), 7.34 (bs, 1 H), 4.37 (m, 2 H), 1.36 (t, *J* = 7.06 Hz, 3 H).

Compound 62: 5-(Benzylamino-methyl)-1H-pyrazole-3-carboxylic acid ethyl ester.



To a 20 mL vial with stirring bar was added 5-formyl-1H-pyrazole-3-carboxylic acid ethyl ester (0.20 g, 1.19 mmol) and 1,2-dichloroethane (3 mL). Benzylamine (0.130 mL, 1.19 mmol) was added, followed by sodium triacetoxyborohydride (0.504 g, 2.37 mmol). The vial was capped with a septum flushed with N<sub>2</sub>, and stirred overnight at

59.US1.PRO

## Provisional Application

room temperature. The mixture was added to  $\text{NaHCO}_3$  (10 mL) and extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 10 mL). The combined organic extracts were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified using preparatory-HPLC providing 0.254 g (87 %) of 5-(benzylamino-methyl)-1H-pyrazole-3-carboxylic acid ethyl ester: LC-MS  $m/z$  260 (M+1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.30 (s, 1 H), 7.60-7.31 (m, 5 H), 6.87 (s, 1 H), 4.22 (q,  $J = 7.1$  Hz, 2 H), 4.11 (m, 4 H), 1.21 (t,  $J = 7.3$  Hz, 3 H).

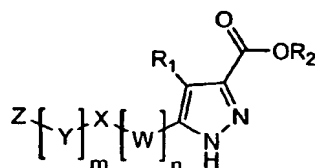
Throughout this application, various publications, patents and published patent applications are cited. The disclosures of these publications, patents and published patent applications referenced in this application are hereby incorporated by reference in their entirety into the present disclosure. Modifications and extension of the disclosed inventions that are within the purview of the skilled artisan are encompassed within the above disclosure and the claims that follow.

Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous human GPCRs, it is most preferred that the vector utilized be pCMV. This vector was deposited with the American Type Culture Collection (ATCC) on October 13, 1998 (10801 University Blvd., Manassas, VA 20110-2209 USA) under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The DNA was tested by the ATCC and determined to be viable. The ATCC has assigned the following deposit number to pCMV: ATCC #203351.

## CLAIMS

What we claim is:

1. A compound of Formula (I):



(I)

wherein:

W and Y are independently a straight or branched chain C<sub>1-5</sub> alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said C<sub>1-5</sub> alkylene group is optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> haloalkyl or C<sub>1-4</sub> alkoxy;

X is -NR<sub>3</sub>C(O)-, -C(O)NR<sub>3</sub>, -NR<sub>3</sub>S(O)<sub>2</sub>-, -S(O)<sub>2</sub>NR<sub>3</sub>-, -NR<sub>3</sub>C(O)NR<sub>4</sub>-, -NR<sub>3</sub>C(O)O-, -OC(O)NR<sub>3</sub>-, -NR<sub>3</sub>-, -C(O)-, -CH(OH)-, -C(NH)-, -O-, -S-, -S(O)- or -S(O)<sub>2</sub>-;

R<sub>3</sub> and R<sub>4</sub> are independently H, C<sub>1-4</sub> alkyl, phenyl or heteroaryl, wherein each of said alkyl, phenyl or heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxyl, thiol, cyano, nitro, C<sub>1-4</sub> haloalkyl, amino, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

Z is H, halogen, phenyl or heteroaryl, wherein said phenyl and heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxy, thiol, cyano, nitro, C<sub>1-4</sub> haloalkyl, amino, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

R<sub>1</sub> is H, hydroxyl, halogen, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> haloalkyl;

R<sub>2</sub> is H or C<sub>1-8</sub> alkyl and

"n" and "m" or independently 0 or 1; or

a pharmaceutically acceptable salt, solvate or hydrate thereof;

provided that:

i) when both  $R_1$  and  $R_2$  are H then  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2H$ ,  $C(O)-C_6H_4-p-O-C_8H_{17}$ ,  $OCH_2CH_3$ ,  $OH$ ,  $CH_2CH_2CH_2CH_2CO_2H$ ,  $CH_2CH_2CH_2CO_2H$ ,  $CH_2CO_2H$  or  $CH_2CH_2CO_2H$ ;

ii) when  $R_1$  is  $CH_3$  and  $R_2$  is H then  $-[W]_n-X-[Y]_m-Z$  together is not  $CH_2CO_2H$ ,  $C(O)CH=CH C_6H_5$ ,  $C(O)C_6H_4-p-OCH_3$ ,  $CO_2H$ ,  $C(O)CH_3$ ,  $C(O)C_6H_4-o-CH_3$ ,  $C(O)C_6H_4-o-Br$ ,  $C(O)C_6H_4-o-Cl$ , or  $C(O)C_6H_5$ ;

iii) when  $R_1$  is Br and  $R_2$  is H then  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2H$ ;

iv) when  $R_1$  is OH and  $R_2$  is H then  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2H$ ;

v) when  $R_1$  is H and  $R_2$  is  $CH_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not 2,6-dichloro-4-trifluoromethylphenoxy,  $C(O)NH-C_6H_4-p-OCH_2CH_3$ ,  $NHC(O)CH(CH_3)_2$ ,  $SCH_3$ ,  $C(O)-C_6H_4-p-O-C_8H_{17}$ ,  $SCH_2CH_3$ ,  $C(O)NHC_6H_5$ ,  $CH(OCH_3)_2$ ,  $CH_2OC(O)CH_3$ ,  $CO_2H$ ,  $CO_2CH_3$ ,  $C(O)C_6H_4-p-NO_2$ ,  $C(O)C_6H_5$ ,  $CH_2CH_2CO_2CH_3$ ,  $CH_2CH_2CH_2CH_2CO_2CH_3$ ,  $CH_2CH_2CH_2CO_2CH_3$  or  $CH_2CO_2CH_3$ ;

vi) when  $R_1$  is OH and  $R_2$  is  $CH_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not  $CH_2OCH_2C_6H_5$ ,  $CH_2OCH(CH_3)_2$  or  $CH_2OH$ ;

vii) when  $R_2$  is  $CH_3$  then:

$R_1$  is not  $CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is not 2,6-dichloro-4-trifluoromethylphenoxy;

$R_1$  is not I and  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2C(CH_3)_3$ ;

$R_1$  is not  $C(CH_3)_3$  and  $-[W]_n-X-[Y]_m-Z$  together is not formyl;

$R_1$  is not Br and  $-[W]_n-X-[Y]_m-Z$  together is not  $CO_2CH_3$ ;

or

$R_1$  is not  $CH_2CH_2CH_2CH_3$  and  $-[W]_n-X-[Y]_m-Z$  together is not formyl;

viii) when  $R_1$  is H and  $R_2$  is  $CH_2CH_3$  then  $-[W]_n-X-[Y]_m-Z$  together is not  $CH_2SCH_2CH_3$ ,  $OCH_2CH_2CH=CH_2$ ,  $CH_2CH_2CH_2OH$ ,  $CH_2CH_2CHO$ ,

## 59.US1.PRO

## Provisional Application

$\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $\text{OCH}_3$ ,  $\text{C}(\text{O})\text{CH}_2\text{Br}$ ,  $\text{CO}_2\text{C}_8\text{H}_{17}$ , formyl,  $\text{OH}$ ,  $\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$ ,  
 $\text{CH}(\text{CH}_3)\text{OC}(\text{O})\text{CH}_3$ ,  $\text{CH}_2\text{OH}$ ,  $\text{CH}_2\text{OC}(\text{O})\text{CH}_3$ ,  $\text{C}(\text{O})\text{CH}_3$ ,  $\text{C}(\text{O})\text{C}_6\text{H}_5$  or  
 $\text{C}(\text{O})\text{NHCH}_2\text{CO}_2\text{CH}_2\text{CH}_3$ .

ix) when  $\text{R}_1$  is  $\text{CH}_3$  and  $\text{R}_2$  is  $\text{CH}_2\text{CH}_3$  then  $-\text{[W]}_n\text{-X-[Y]}_m\text{-Z}$  together  
 is not  $\text{CH}(\text{OH})\text{C}_6\text{H}_4\text{-}p\text{-N}(\text{Cl})_2$ ,  $\text{C}(\text{O})\text{Cl}_2\text{C}(\text{O})\text{CH}_3$ ,  $\text{CO}_2\text{CH}_2\text{C}_6\text{H}_5$ ,  $\text{CO}_2\text{CH}_3$ ,  
 $\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_3$ ,  $\text{C}(\text{O})\text{CH}_3$ ,  $\text{C}(\text{O})\text{C}_6\text{H}_4\text{-}p\text{-OCH}_3$ ,  $\text{C}(\text{O})\text{C}_6\text{H}_4\text{-}o\text{-Br}$ ,  $\text{C}(\text{O})\text{C}_6\text{H}_4\text{-}p\text{-}$   
 $\text{Cl}$ ,  $\text{C}(\text{O})\text{C}_6\text{H}_4\text{-}o\text{-Cl}$ ,  $\text{C}(\text{O})\text{CH}_2\text{C}_6\text{H}_5$  or  $\text{C}(\text{O})\text{C}_6\text{H}_5$ ;

x) when  $\text{R}_2$  is  $\text{CH}_2\text{CH}_3$  then:

$\text{R}_1$  is not  $\text{I}$  and  $-\text{[W]}_n\text{-X-[Y]}_m\text{-Z}$  together is not  
 $\text{CO}_2\text{CH}_2\text{CH}_3$ ;

$\text{R}_1$  is not  $\text{CF}_3$  and  $-\text{[W]}_n\text{-X-[Y]}_m\text{-Z}$  together is not  
 $\text{CO}_2\text{CH}_2\text{CH}_3$ ; or

$\text{R}_1$  is not  $\text{Br}$  and  $-\text{[W]}_n\text{-X-[Y]}_m\text{-Z}$  together is not  
 $\text{CO}_2\text{CH}_2\text{CH}_3$ ;

xi) when  $\text{R}_1$  is  $\text{OH}$  and  $\text{R}_2$  is  $\text{CH}_2\text{CH}_3$  then  $-\text{[W]}_n\text{-X-[Y]}_m\text{-Z}$  together  
 is not  $\text{C}(\text{O})\text{C}_6\text{H}_5$ ,  $\text{C}(\text{O})\text{NH}_2$  or  $\text{CO}_2\text{CH}_2\text{CH}_3$ ;

xii) when  $\text{R}_1$  is  $\text{H}$  and  $\text{R}_2$  is  $\text{C}(\text{CH}_3)_3$  then  $-\text{[W]}_n\text{-X-[Y]}_m\text{-Z}$  together is  
 not  $\text{CO}_2\text{C}(\text{CH}_3)_3$ ,  $\text{C}(\text{O})\text{NHC}(\text{O})\text{CH}_3$  or  $\text{C}(\text{O})\text{NH}_2$ ;

xiii) when  $\text{R}_1$  is  $\text{OH}$  and  $\text{R}_2$  is  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$  then  $-\text{[W]}_n\text{-X-[Y]}_m\text{-Z}$   
 together is not  $\text{C}(\text{O})\text{C}_6\text{H}_5$ ; or

xiv) when  $\text{X}$  is  $-\text{NR}_3-$  then "n" is not 0.

2. The compound according to claim 1 wherein "n" is 0.

3. The compound according to claim 1 wherein "n" is 1.

4. The compound according to any one of claims 1 to 3 wherein "m" is 0.

5. The compound according to any one of claims 1 to 3 wherein "m" is 1.

6. The compound according to any one of claims 1, 3, 4 and 5 wherein  $\text{W}$  is the  
 straight or branched  $\text{C}_{1-5}$  alkylene group optionally containing one double bond,  
 one triple bond or carbonyl, wherein said  $\text{C}_{1-5}$  alkylene group is optionally  
 substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

## 59.US1.PRO

## Provisional Application

7. The compound according to claim 6 wherein W is  $-\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
- 5 8. The compound according to claim 7 wherein W is  $-\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl or  $\text{C}_{1-4}$  alkoxy.
9. The compound according to claim 7 wherein W is  $-\text{C}(\text{CH}_3)_2-$ .
- 10 10. The compound according to claim 6 wherein W is  $-\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
11. The compound according to claim 10 wherein W is  $-\text{CH}(\text{CH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
- 15 12. The compound according to claim 10 wherein W is  $-\text{C}(\text{CH}_3)_2\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{CH}_3)_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
- 20 13. The compound according to claim 10 wherein W is  $-\text{CH}(\text{OCH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{OCH}_3)-$  optionally substituted with halogen, hydroxyl or  $\text{C}_{1-4}$  alkyl.
- 25 14. The compound according to claim 6 wherein W is  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
- 30 15. The compound according to claim 6 wherein W is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
16. The compound according to claim 6 wherein W is  $-\text{CH}-\text{CH}-$  optionally substituted with  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
17. The compound according to claim 6 wherein W is  $-\text{C}\equiv\text{C}-$ .

59.US1.PRO

Provisional Application

18. The compound according to claim 6 wherein W is -C(O)-.
19. The compound according to claim 6 wherein W is -CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.  
5
20. The compound according to claim 19 wherein W is -CH(CH<sub>3</sub>)C(O)- or -C(O)CH(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
- 10 21. The compound according to claim 19 wherein W is -C(CH<sub>3</sub>)<sub>2</sub>C(O)- or -C(O)C(CH<sub>3</sub>)<sub>2</sub>-.
22. The compound according to claim 6 wherein W is -CH<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.  
15
23. The compound according to claim 22 wherein W is -C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>C(CH<sub>3</sub>)<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.  
20
24. The compound according to claim 6 wherein W is -CH<sub>2</sub>C(O)CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
25. The compound according to claim 6 wherein W is -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.  
25
26. The compound according to claim 6 wherein W is -CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>CH<sub>2</sub>CH(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.  
30
27. The compound according to claim 6 wherein W is -CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>- or -CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.

59.US1.PRO

## Provisional Application

28. The compound according to claim 6 wherein W is  $-\text{CH}=\text{CHC}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}=\text{CH}-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
29. The compound according to claim 6 wherein W is  $-\text{C}(\text{CH}_3)=\text{CHC}(\text{O})-$  or  $-\text{C}(\text{O})\text{CH}=\text{C}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
30. The compound according to any one of claims 1, 2, 3 and 5 to 28 wherein Y is the straight or branched chain  $\text{C}_{1-5}$  alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said  $\text{C}_{1-5}$  alkylene group is optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
31. The compound according to claim 30 wherein Y is  $-\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
32. The compound according to claim 31 wherein Y is  $-\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl or  $\text{C}_{1-4}$  alkoxy.
33. The compound according to claim 31 wherein Y is  $-\text{C}(\text{CH}_3)_2-$ .
34. The compound according to claim 30 wherein Y is  $-\text{CH}_2\text{CH}_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
35. The compound according to claim 34 wherein Y is  $-\text{CH}(\text{CH}_3)\text{CH}_2-$  or  $-\text{CH}_2\text{CH}(\text{CH}_3)-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
36. The compound according to claim 34 wherein Y is  $-\text{C}(\text{CH}_3)_2\text{CH}_2-$  or  $-\text{CH}_2\text{C}(\text{CH}_3)_2-$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.
37. The compound according to claim 34 wherein Y is  $-\text{CH}(\text{OCH}_3)\text{CH}_2-$  or

59.US1.PRO

## Provisional Application

-CH<sub>2</sub>CH(OCH<sub>3</sub>)- optionally substituted with halogen, hydroxyl or C<sub>1-4</sub> alkyl.

38. The compound according to claim 30 wherein Y is -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
39. The compound according to claim 30 wherein Y is -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
40. The compound according to claim 30 wherein Y is -CH=CH- optionally substituted with C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
41. The compound according to claim 30 wherein Y is -C≡C-.
42. The compound according to claim 30 wherein Y is -C≡CCH<sub>2</sub>- or -CH<sub>2</sub>C≡C- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
43. The compound according to claim 30 wherein Y is -C(O)-.
44. The compound according to claim 30 wherein Y is -CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
45. The compound according to claim 44 wherein Y is -CH(CH<sub>3</sub>)C(O)- or -C(O)CH(CH<sub>3</sub>)- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
46. The compound according to claim 44 wherein Y is -C(CH<sub>3</sub>)<sub>2</sub>C(O)- or -C(O)C(CH<sub>3</sub>)<sub>2</sub>-.
47. The compound according to claim 30 wherein Y is -CH<sub>2</sub>CH<sub>2</sub>C(O)- or -C(O)CH<sub>2</sub>CH<sub>2</sub>- optionally substituted with halogen, hydroxyl, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.
48. The compound according to claim 47 wherein Y is -C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>C(O)- or

59.US1.PRO

Provisional Application

$\text{-C(O)CH}_2\text{C(CH}_3)_2\text{-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

5 49. The compound according to claim 30 wherein Y is  $\text{-CH}_2\text{C(O)CH}_2\text{-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

10 50. The compound according to claim 30 wherein Y is  $\text{-CH}_2\text{CH}_2\text{CH}_2\text{C(O)-}$  or  $\text{-C(O)CH}_2\text{CH}_2\text{CH}_2\text{-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

51. The compound according to claim 30 wherein Y is  $\text{-CH(CH}_3\text{)CH}_2\text{CH}_2\text{C(O)-}$  or  $\text{-C(O)CH}_2\text{CH}_2\text{CH(CH}_3\text{)-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

15 52. The compound according to claim 30 wherein Y is  $\text{-CH}_2\text{CH}_2\text{C(O)CH}_2\text{-}$  or  $\text{-CH}_2\text{C(O)CH}_2\text{CH}_2\text{-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

20 53. The compound according to claim 30 wherein Y is  $\text{-CH=CHC(O)-}$  or  $\text{-C(O)CH=CH-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

25 54. The compound according to claim 30 wherein Y is  $\text{-C(CH}_3\text{)=CHC(O)-}$  or  $\text{-C(O)CH=C(CH}_3\text{)-}$  optionally substituted with halogen, hydroxyl,  $\text{C}_{1-4}$  alkyl or  $\text{C}_{1-4}$  alkoxy.

55. The compound according to any one of claims 1 to 54 wherein X is  $\text{-NR}_3\text{C(O)-}$ .

30 56. The compound according to any one of claims 1 to 54 wherein X is  $\text{-C(O)NR}_3\text{-}$ .

57. The compound according to any one of claims 1 to 54 wherein X is  $\text{-NR}_3\text{S(O)}_2\text{-}$ .

58. The compound according to any one of claims 1 to 54 wherein X is

59.US1.PRO

## Provisional Application

-S(O)<sub>2</sub>NR<sub>3</sub>-.

59. The compound according to any one of claims 1 to 54 wherein X is  
-NR<sub>3</sub>C(O)NR<sub>4</sub>-.

5

60. The compound according to any one of claims 1 to 54 wherein X is -NR<sub>3</sub>C(O)O-

61. The compound according to any one of claims 1 to 54 wherein X is -OC(O)NR<sub>3</sub>-

10

62. The compound according to any one of claims 1 to 54 wherein X is -NR<sub>3</sub>-.

63. The compound according to any one of claims 55 to 62 wherein R<sub>3</sub> is H or CH<sub>3</sub>.

15

64. The compound according to claim 59 wherein R<sub>4</sub> is H or CH<sub>3</sub>.

65. The compound according to any one of claims 1 to 54 wherein X is -C(O)-.

20

66. The compound according to any one of claims 1 to 54 wherein X is -CH(OH)-.

67. The compound according to any one of claims 1 to 54 wherein X is -C(NH)-.

68. The compound according to any one of claims 1 to 54 wherein X is -O-.

25

69. The compound according to any one of claims 1 to 54 wherein X is -S-.

70. The compound according to any one of claims 1 to 54 wherein X is -S(O)-.

30

71. The compound according to any one of claims 1 to 54 wherein X is -S(O)<sub>2</sub>-.

72. The compound according to any one of claims 1 to 71 wherein Z is H.

73. The compound according to any one of claims 1 to 71 wherein Z is halogen.

59.US1.PRO

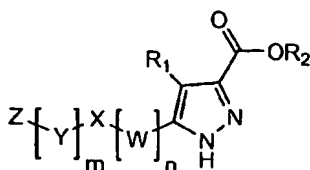
## Provisional Application

74. The compound according to any one of claims 1 to 71 wherein Z is phenyl.
75. The compound according to claim 74 wherein the phenyl is optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl.
76. The compound according to claim 75 wherein the phenyl is optionally substituted with 1 to 3 substituents selected from the group consisting of -F, -Cl, -Br, -CF<sub>3</sub>, -NHCH<sub>3</sub>, -N(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, -OCH<sub>3</sub> and -OCF<sub>3</sub>.
77. The compound according to any one of claims 1 to 71 wherein Z is heteroaryl.
78. The compound according to claim 77 wherein the heteroaryl is optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, C<sub>1-4</sub> haloalkyl, C<sub>1-4</sub> aminoalkyl, C<sub>2-4</sub> aminodialkyl, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl.
79. The compound according to claim 78 wherein the phenyl is optionally substituted with 1 to 3 substituents selected from the group consisting of -F, -Cl, -Br, -CF<sub>3</sub>, -NHCH<sub>3</sub>, -N(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, -OCH<sub>3</sub> and -OCF<sub>3</sub>.
80. The compound according to any one of claims 1 to 79 wherein R<sub>1</sub> is H.
81. The compound according to any one of claims 1 to 79 wherein R<sub>1</sub> is hydroxyl.
82. The compound according to any one of claims 1 to 78 wherein R<sub>1</sub> is halogen.
83. The compound according to any one of claims 1 to 78 wherein R<sub>1</sub> is C<sub>1-4</sub> alkyl.

59.US1.PRO

## Provisional Application

84. The compound according to any one of claims 1 to 78 wherein  $R_1$  is  $C_{1-4}$  haloalkyl.
85. The compound according to any one of claims 1 to 84 wherein  $R_2$  is H.
86. The compound according to any one of claims 1 to 84 wherein  $R_2$  is  $C_{1-8}$  alkyl.
87. A pharmaceutical composition comprising a pharmaceutically acceptable carrier in combination with at least one compound according to Formula (I):



(I)

wherein:

W and Y are independently a straight or branched chain  $C_{1-5}$  alkylene group optionally containing one double bond, one triple bond or carbonyl, wherein said  $C_{1-5}$  alkylene group is optionally substituted with halogen, hydroxyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  haloalkyl or  $C_{1-4}$  alkoxy;

X is  $-NR_3C(O)-$ ,  $-C(O)NR_3$ ,  $-NR_3S(O)_2-$ ,  $-S(O)_2NR_3-$ ,  $-NR_3C(O)NR_4-$ ,  $-NR_3C(O)O-$ ,  $-OC(O)NR_3-$ ,  $-NR_3-$ ,  $-C(O)-$ ,  $-CH(OH)-$ ,  $-C(NH)-$ ,  $-O-$ ,  $-S-$ ,  $-S(O)-$  or  $-S(O)_2-$ ;

$R_3$  and  $R_4$  are independently H,  $C_{1-4}$  alkyl, phenyl or heteroaryl, wherein each of said alkyl, phenyl or heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxyl, thiol, cyano, nitro,  $C_{1-4}$  haloalkyl, amino,  $C_{1-4}$  aminoalkyl,  $C_{2-4}$  aminodialkyl,  $C_{1-4}$  alkyl,  $C_{1-4}$  alkoxy,  $C_{2-4}$  alkenyl,  $C_{2-4}$  alkynyl,  $C_{1-4}$  haloalkoxy,  $C_{1-4}$  alkylthio,  $C_{1-4}$  alkylsulfinyl,  $C_{1-4}$  alkylsulfonyl,  $C_{1-4}$  haloalkylthio,  $C_{1-4}$  haloalkylsulfinyl and  $C_{1-4}$  haloalkylsulfonyl;

Z is H, halogen, phenyl or heteroaryl, wherein said phenyl and heteroaryl are optionally substituted with 1 to 5 substituents selected from the group consisting of halogen, hydroxy, thiol, cyano, nitro,  $C_{1-4}$  haloalkyl, amino,  $C_{1-4}$  aminoalkyl,  $C_{2-4}$  aminodialkyl,  $C_{1-4}$  alkyl,  $C_{1-4}$

## 59.US1.PRO

## Provisional Application

alkoxy, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>1-4</sub> haloalkoxy, C<sub>1-4</sub> alkylthio, C<sub>1-4</sub> alkylsulfinyl, C<sub>1-4</sub> alkylsulfonyl, C<sub>1-4</sub> haloalkylthio, C<sub>1-4</sub> haloalkylsulfinyl and C<sub>1-4</sub> haloalkylsulfonyl;

R<sub>1</sub> is H, hydroxyl, halogen, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> haloalkyl;

R<sub>2</sub> is H or C<sub>1-8</sub> alkyl and

"n" and "m" or independently 0 or 1; or

a pharmaceutically acceptable salt, solvate or hydrate thereof.

88. The pharmaceutical composition according to claim 87 further comprising one or more agents selected from the group consisting of  $\alpha$ -glucosidase inhibitor, aldose reductase inhibitor, biguanide, HMG-CoA reductase inhibitor, squalene synthesis inhibitor, fibrate, LDL catabolism enhancer, angiotensin converting enzyme inhibitor, insulin secretion enhancer and thiazolidinedione.
89. The pharmaceutical composition according to claim 88 wherein the agent is a  $\alpha$ -glucosidase inhibitor.
90. The pharmaceutical composition according to claim 89 wherein the  $\alpha$ -glucosidase inhibitor is acarbose, voglibose or miglitol.
91. The pharmaceutical composition according to claim 90 wherein the  $\alpha$ -glucosidase inhibitor is voglibose.
92. The pharmaceutical composition according to claim 88 wherein the agent is an aldose reductase inhibitor.
93. The pharmaceutical composition according to claim 92 wherein the aldose reductase inhibitor is tolurestat; epalrestat; imirestat; zenarestat; zopolrestat; or sorbinil.
94. The pharmaceutical composition according to claim 88 wherein the agent is a biguanide.

59.US1.PRO

Provisional Application

95. The pharmaceutical composition according to claim 94 wherein the biguanide is phenformin, metformin or buformin.
- 5 96. The pharmaceutical composition according to claim 95 wherein the biguanide is metformin.
97. The pharmaceutical composition according to claim 88 wherein the agent is a HMG-CoA reductase inhibitor.
- 10 98. The pharmaceutical composition according to claim 97 wherein the HMG-CoA reductase inhibitor is rosuvastatin, pravastatin, simvastatin, lovastatin, atorvastatin, fluvastatin or cerivastatin.
- 15 99. The pharmaceutical composition according to claim 88 wherein the agent is a fibrate.
100. The pharmaceutical composition according to claim 99 wherein the fibrate is bezafibrate, bezafibrate, binifibrate, ciprofibrate, ciprofibrate, clofibrate, clofibric acid, etofibrate, fenofibrate, gemfibrozil, nicofibrate, pirifibrate, ronifibrate, 20 simfibrate, or theofibrate.
101. The pharmaceutical composition according to claim 88 wherein the agent is an angiotensin converting enzyme inhibitor.
- 25 102. The pharmaceutical composition according to claim 101 wherein the angiotensin converting enzyme inhibitor is captopril, enalapril, alacepril, delapril; ramipril, lisinopril, imidapril, benazepril, ceronapril, cilazapril, enalaprilat, fosinopril, moxeltopril, perindopril, quinapril, spirapril, temocapril or trandolapril.
- 30 103. The pharmaceutical composition according to claim 88 wherein the agent is an insulin secretion enhancer.
104. The pharmaceutical composition according to claim 103 wherein the insulin secretion enhancer is tolbutamide; chlorpropamide; tolazamide; acetohexamide;

59.US1.PRO

## Provisional Application

glycopyramide; glibenclamide; gliclazide; 1-butyl-3-metanyllurea; carbutamide; glibonuride; glipizide; gliquidone; glisoxepid; glybuthiazole; glibuzole; glyhexamide; glymidine; glypinamide; phenbutamide; tolcyclamide, glimepiride, nateglinide, or mitiglinide.

5

105. The pharmaceutical composition according to claim 88 wherein the agent is a thiazolidinedione.

10

106. The pharmaceutical composition according to claim 105 wherein the thiazolidinedione is rosiglitazone or pioglitazone.

107. The pharmaceutical composition according to claim 106 wherein the thiazolidinedione is rosiglitazone.

15

108. The compound according to any one of claims 1 to 86 for use in a method of treatment of the human or animal body by therapy.

20

109. The compound according to any one of claims 1 to 86 for use in a method of prophylaxis or treatment of a metabolic-related disorder of the human or animal body by therapy.

25

110. A method for prophylaxis or treatment of a metabolic-related disorder in an individual in need of said prophylaxis or treatment comprising administering to the individual a therapeutically effective amount of a compound according to any one of claims 1 to 86 or a pharmaceutical composition according to any one of claims 87 to 107.

30

111. A method of modulating a RUP25 receptor in an individual comprising contacting the receptor with a compound according to any one of claims 1 to 86.

112. The method of modulating the RUP25 receptor according to claim 111 wherein the compound is an agonist.

59.US1.PRO

Provisional Application

113. The method of modulating the RUP25 receptor according to claim 111 or 112 wherein the modulation of the RUP25 receptor is for prophylaxis or treatment of a metabolic-related disorder in an individual in need of said prophylaxis or treatment.
114. The method according to claim 110 or 113 wherein the metabolic-related disorder is selected from the group consisting of dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance, obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, heart disease and type 2 diabetes.
115. The method according to claim 114 wherein the metabolic-related disorder is dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance and type 2 diabetes.
116. The method according to claim 115 wherein the metabolic-related disorder is dyslipidemia.
117. The method according to claim 115 wherein the metabolic-related disorder is atherosclerosis.
118. The method according to claim 115 wherein the metabolic-related disorder is coronary heart disease.
119. The method according to claim 115 wherein the metabolic-related disorder is insulin resistance.
120. The method according to claim 115 wherein the metabolic-related disorder is type 2 diabetes.
121. Use of a compound according to any one of claims 1 to 86 for production of a medicament for use in prophylaxis or treatment of a metabolic-related disorder.

## 59.US1.PRO

## Provisional Application

- 5      122.    The use according to claim 121 further comprising one or more agents selected from the group consisting of  $\alpha$ -glucosidase inhibitor, aldose reductase inhibitor, biguanide, HMG-CoA reductase inhibitor, squalene synthesis inhibitor, fibrate, LDL catabolism enhancer, angiotensin converting enzyme inhibitor, insulin secretion enhancer and thiazolidinedione.
- 10      123.    The use according to claim 122 wherein the agent is a  $\alpha$ -glucosidase inhibitor.
124.    The use according to claim 123 wherein the  $\alpha$ -glucosidase inhibitor is acarbose, voglibose or miglitol.
125.    The use according to claim 124 wherein the  $\alpha$ -glucosidase inhibitor is voglibose.
- 15      126.    The use according to claim 122 wherein the agent is an aldose reductase inhibitor.
127.    The use according to claim 126 wherein the aldose reductase inhibitor is tolurestat; epalrestat; imirestat; zenarestat; zopolrestat; or sorbinil.
- 20      128.    The use according to claim 122 wherein the agent is a biguanide.
129.    The use according to claim 128 wherein the biguanide is phenformin, metformin or buformin.
- 25      130.    The use according to claim 129 wherein the biguanide is metformin.
131.    The use according to claim 122 wherein the agent is a HMG-CoA reductase inhibitor.
- 30      132.    The use according to claim 131 wherein the HMG-CoA reductase inhibitor is rosuvastatin, pravastatin, simvastatin, lovastatin, atorvastatin, fluvastatin or cerivastatin.

59.US1.PRO

## Provisional Application

133. The use according to claim 122 wherein the agent is a fibrate.
134. The use according to claim 133 wherein the fibrate is bezafibrate, beclobate,  
binifibrate, ciplofibrate, clinofibrate, clofibrate, clofibric acid, etofibrate,  
5 fenofibrate, gemfibrozil, nicofibrate, pirifibrate, ronifibrate, simfibrate, or  
theofibrate.
135. The use according to claim 122 wherein the agent is an angiotensin converting  
enzyme inhibitor.
- 10 136. The use according to claim 135 wherein the angiotensin converting enzyme  
inhibitor is captopril, enalapril, alacepril, delapril; ramipril, lisinopril, imidapril,  
benazepril, ceronapril, cilazapril, enalaprilat, fosinopril, moveltopril, perindopril,  
quinapril, spirapril, temocapril or trandolapril.
- 15 137. The use according to claim 122 wherein the agent is an insulin secretion  
enhancer.
138. The use according to claim 137 wherein the insulin secretion enhancer is  
20 tolbutamide; chlorpropamide; tolazamide; acetohexamide; glycopyramide;  
glibenclamide; gliclazide; 1-butyl-3-metanilylurea; carbutamide; glibonuride;  
glipizide; gliquidone; glisoxepid; glybuthiazole; glibuzole; glyhexamide;  
glymidine; glypinamide; phenbutamide; tolcyclamide; glimepiride, nateglinide,  
or mitiglinide.
- 25 139. The use according to claim 122 wherein the agent is a thiazolidinedione.
140. The use according to claim 139 wherein the thiazolidinedione is rosiglitazone or  
pioglitazone.
- 30 141. The use according to claim 140 wherein the thiazolidinedione is rosiglitazone.
142. The use according to any one of claims 121 to 141 wherein the metabolic-related  
disorder is dyslipidemia, atherosclerosis, coronary heart disease, insulin

59.US1.PRO

## Provisional Application

resistance, obesity, impaired glucose tolerance, atheromatous disease,  
hypertension, stroke, Syndrome X, heart disease and type 2 diabetes.

5 143. The use according to claim 142 wherein the metabolic-related disorder is  
dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance and type  
2 diabetes.

10 144. The use according to claim 143 wherein the metabolic-related disorder is  
dyslipidemia.

145. The use according to claim 143 wherein the metabolic-related disorder is  
atherosclerosis.

15 146. The use according to claim 143 wherein the metabolic-related disorder is  
coronary heart disease.

147. The use according to claim 143 wherein the metabolic-related disorder is insulin  
resistance.

20 148. The use according to claim 143 wherein the metabolic-related disorder is type 2  
diabetes.

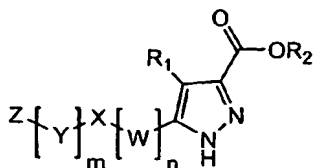
25 149. The method of producing a pharmaceutical composition comprising admixing at  
least one compound according to any one of claims 1 to 86 and a  
pharmaceutically acceptable carrier or excipient.

59.US1.PRO

## Provisional Application

## ABSTRACT

The present invention relates to certain pyrazole carboxylic acid and ester derivatives of Formula (I), and pharmaceutically acceptable salts thereof, which exhibit useful pharmaceutical properties, for example, as agonists for the nicotinic acid receptor.



(I)

Also provided by the present invention are pharmaceutical compositions containing compounds of the invention, and methods of using the compounds and compositions of the invention in the prophylaxis or treatment of metabolic-related disorders, including dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance, type 2 diabetes, Syndrome-X and the like. In addition, the present invention also provides for the use of the compounds of the invention in combination with other active agents such as those belonging to the class of  $\alpha$ -glucosidase inhibitors, aldose reductase inhibitors, biguanides, HMG-CoA reductase inhibitors, squalene synthesis inhibitors, fibrates, LDL catabolism enhancers, angiotensin converting enzyme (ACE) inhibitors, insulin secretion enhancers and the like.

TISSUE

AVG 84

MIN 102

MAX 65

STDDEV 48

STDERR

N

**Figure 2**  
**RUP25 G<sub>i</sub> - coupled constitutive activity in melanophore**

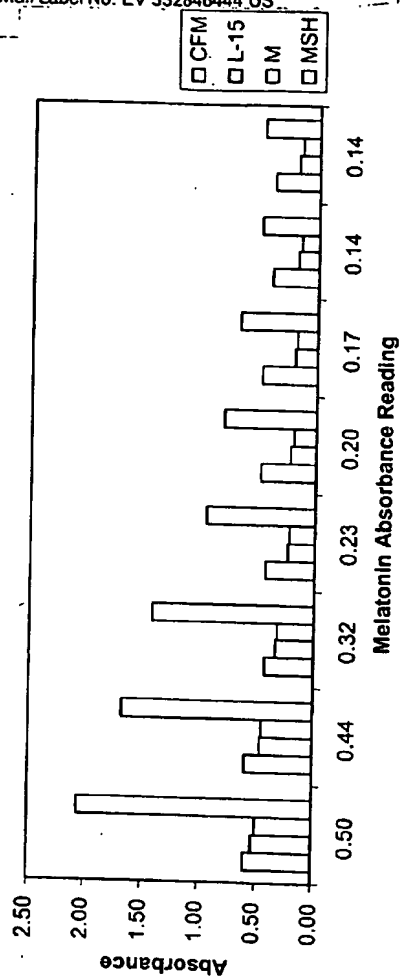
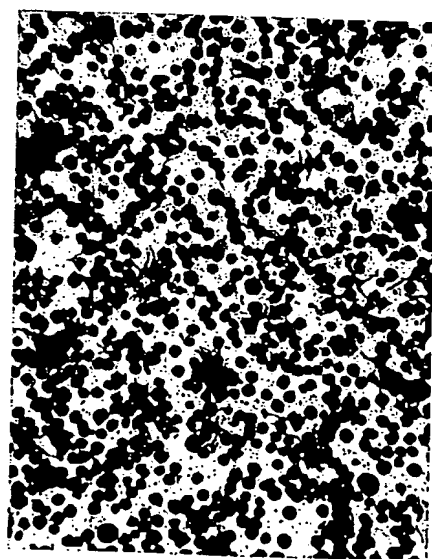


Figure 3A

Action of Nicotinic Acid at RUP25  
Expressing Melanophores

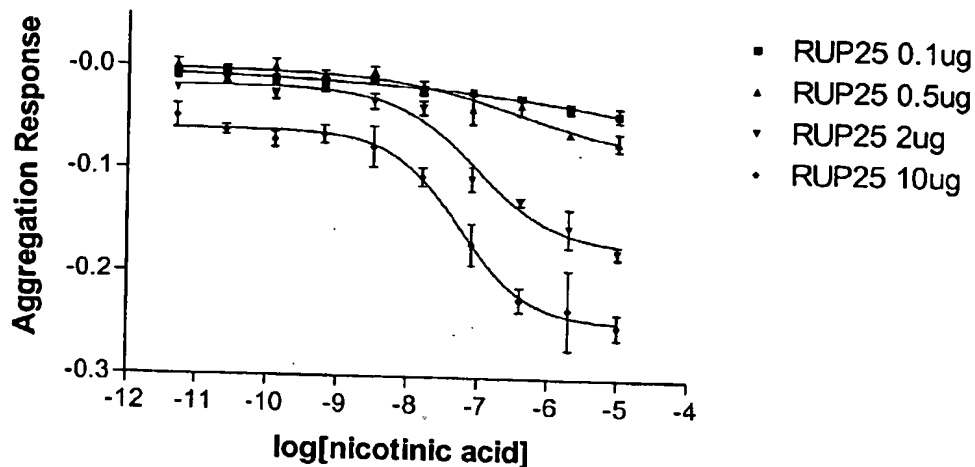
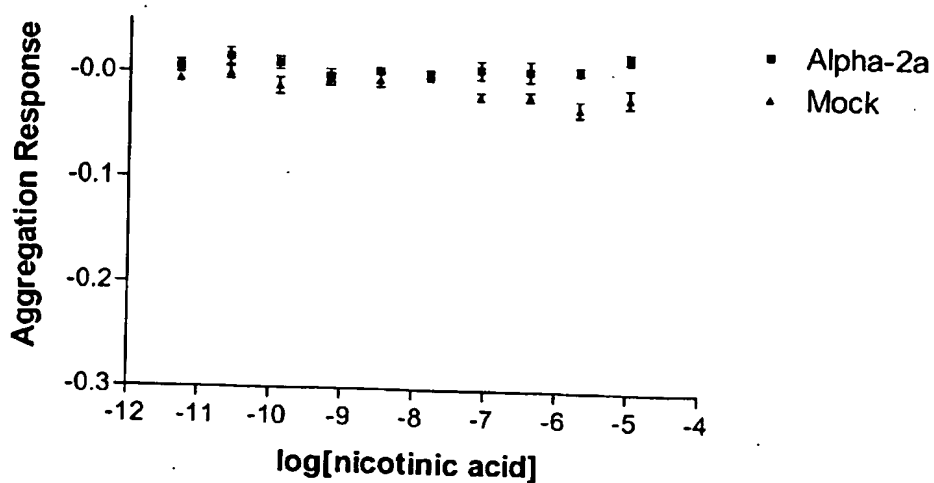


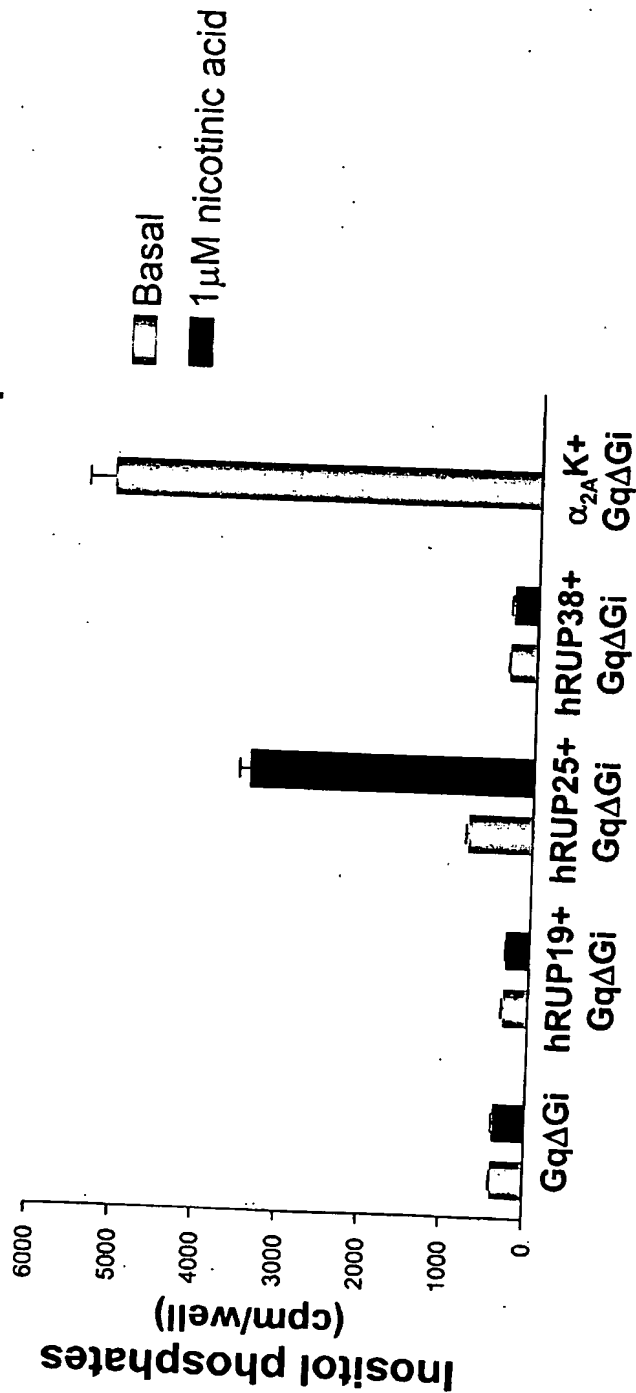
Figure 3B

Nicotinic Acid Control Cells

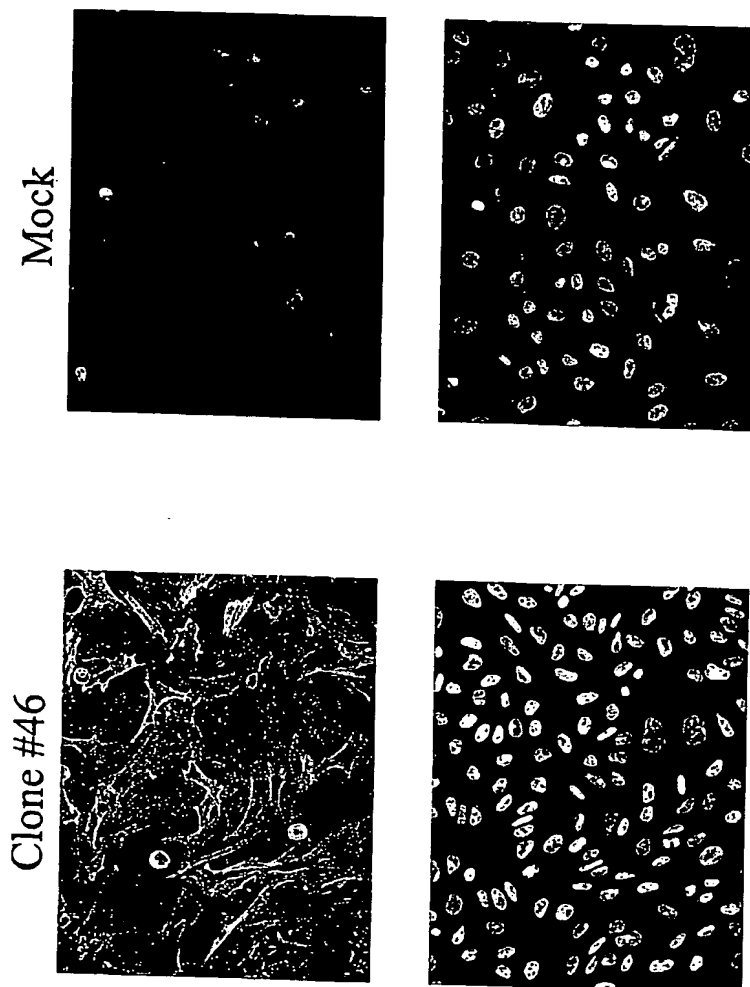


60478664 061303

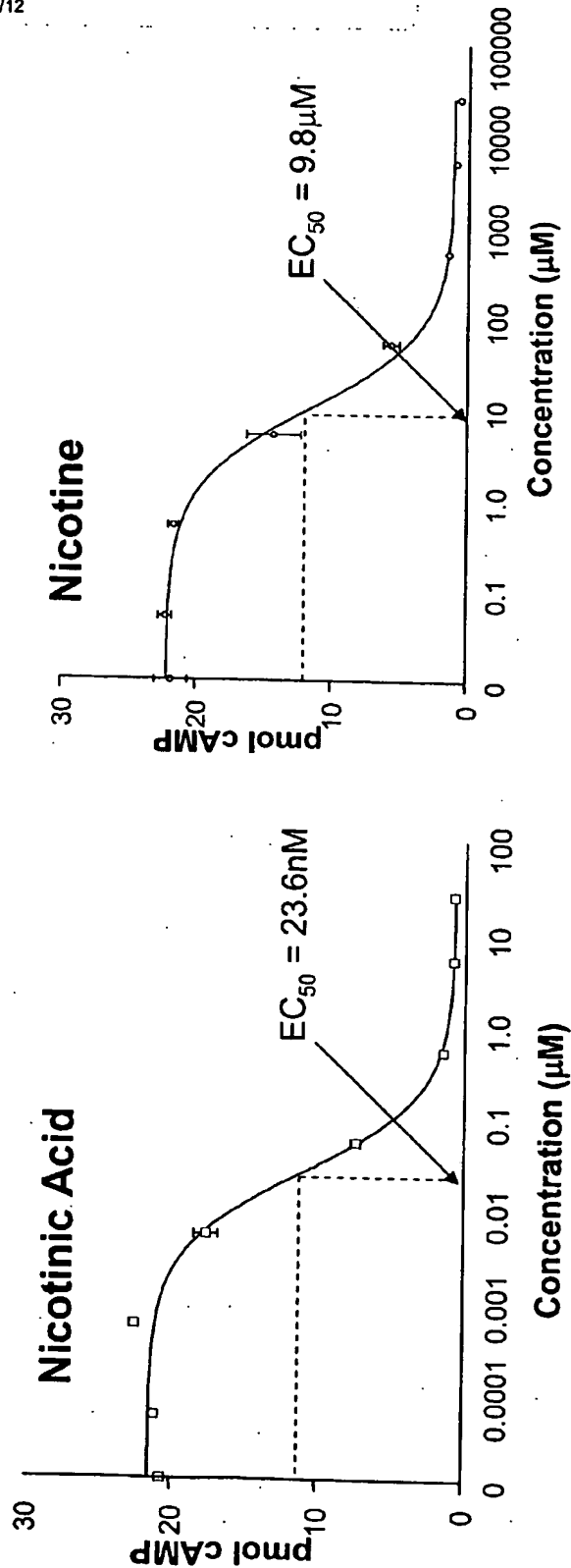
**Figure 4**  
**Nicotinic acid induced-IPs accumulation in 293**  
**cells co-expressing hRUP25 and GqΔGi**



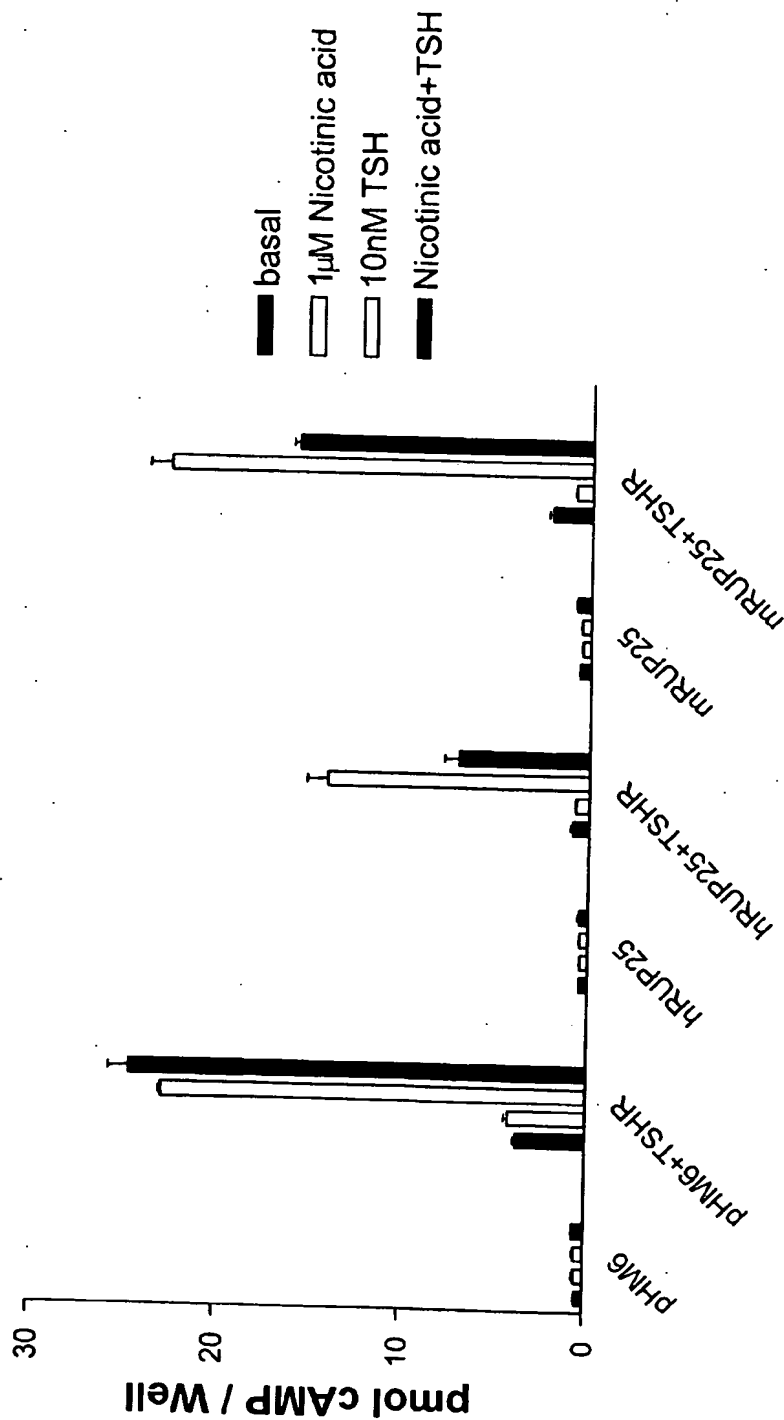
**Figure 5A**  
**hRUP25-CHO stable clone identified by anti-HA**  
**immunofluorescence staining**



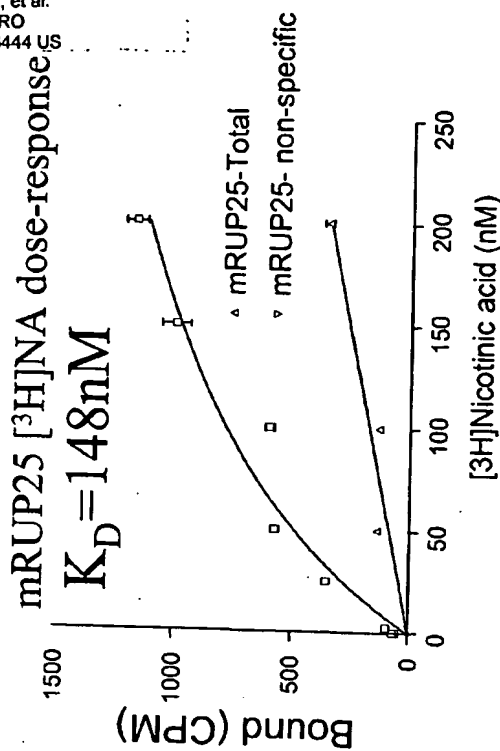
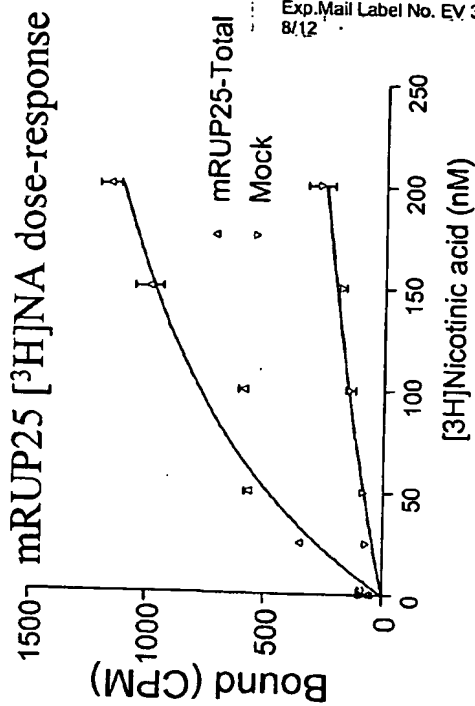
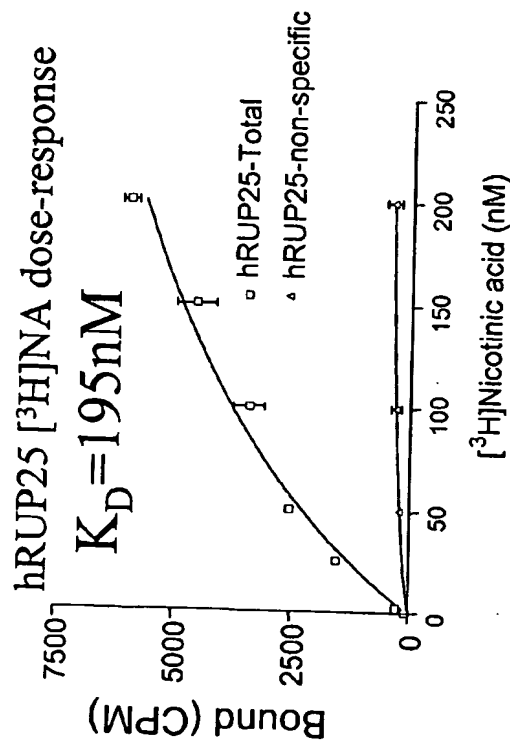
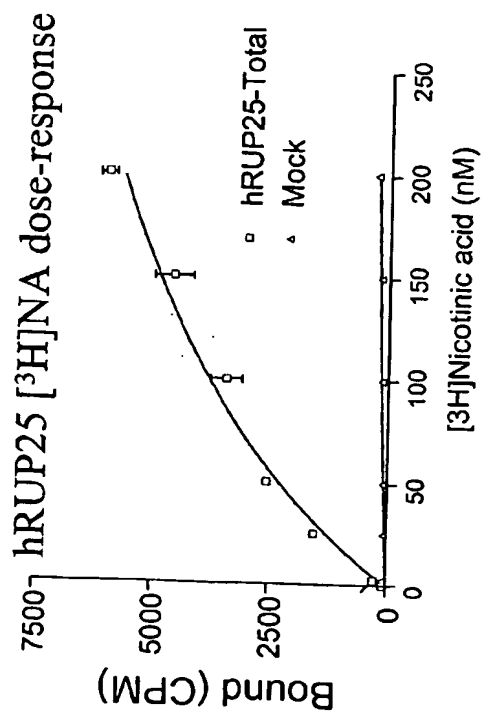
**Figure 5B**  
**Nicotinic acid and nicotine induced-inhibition of**  
**forskolin stimulated cAMP accumulation in**  
**hRUP25-CHO cell stable line #46**



**Figure 6**  
**hRUP25 and mRUP25 inhibit TSHR induced-cAMP**  
**accumulation following activation by nicotinic acid**



**Figure 7**  
**hRUP25 and mRUP25 bind to nicotinic acid**  
**specifically and with high affinity**



## Figure 8

The rank order of potency of compounds on hRUP25 closely matches that of the pharmacologically defined nicotinic acid receptor

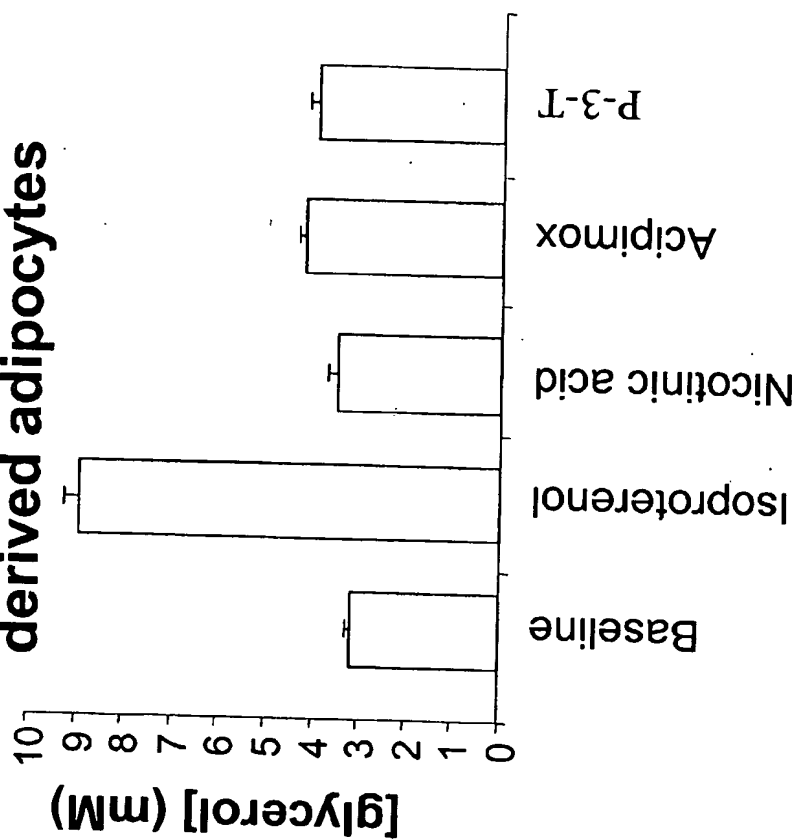
Compound	EC 50 ( $\mu$ M)			
	Adipocytes*	Spleen*	hRUP25†	hRUP25 (K <sub>d</sub> )‡
Nicotinic acid	1.42	0.703	0.04	0.14
Pyridazine-4-carboxylic acid	3.76	3.14	N.D.	2.19
Acipimox	10.3	6.56	2	2.68
3-Pyridine-acetic acid	16.4	21.8	3	1.64
Pyrazine-2-carboxylic acid	26	22	4	4.14
5-Methylnicotinic acid	30.2	30.0	7	3.58
5-Methylpyrazine-2-carboxylic acid	52.0	14.5	7	7.36
6-Methylnicotinic acid	72.6	53.7	34	21.95
Nicotinic acid-1-oxide	80.4	73.7	120	55.25
2-Hydroxynicotinic acid	132	N.D.	130	145.4
Furane-3-carboxylic acid	142	N.D.	110	130.6
Nicotinamide	>1000	>1000	>1000	128.2
N.D., not determined.				

\* From Lorenzen, A. et al. *Mol. Pharmacol.* 59 (2):349-357, 2001.

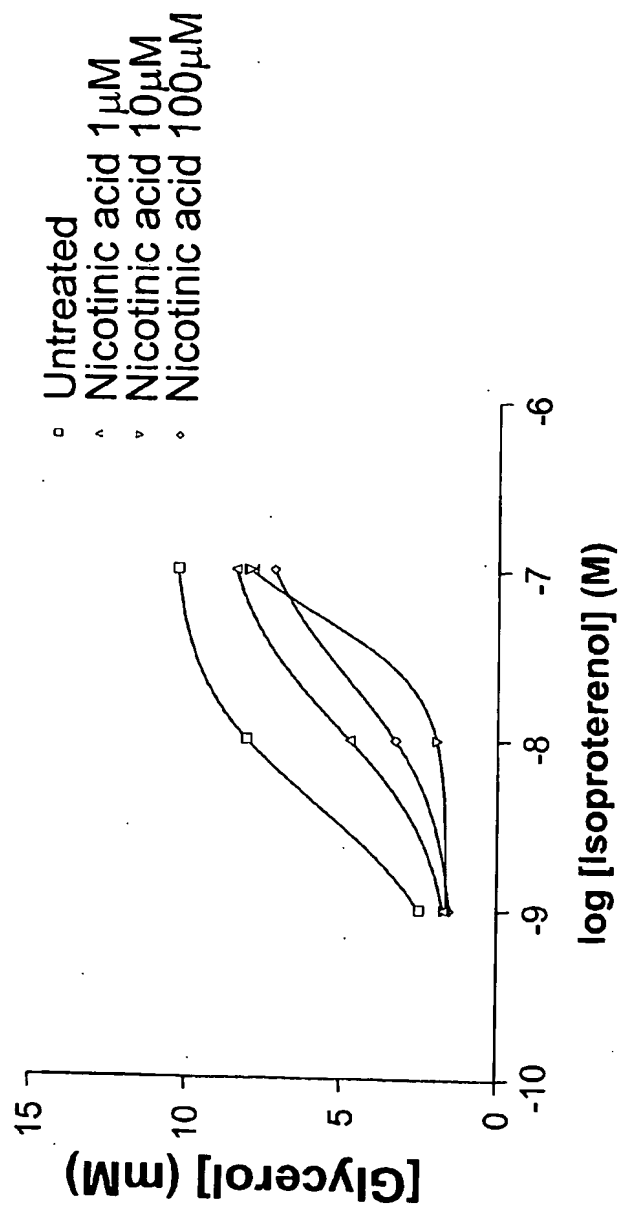
† Arena data, inhibition of forskolin-induced cAMP production in hRUP25-CHO stable line #46.

‡ Arena data, [<sup>3</sup>H]nicotinic acid radioligand binding assay on membranes derived from hRUP25-CHO stable line #46.

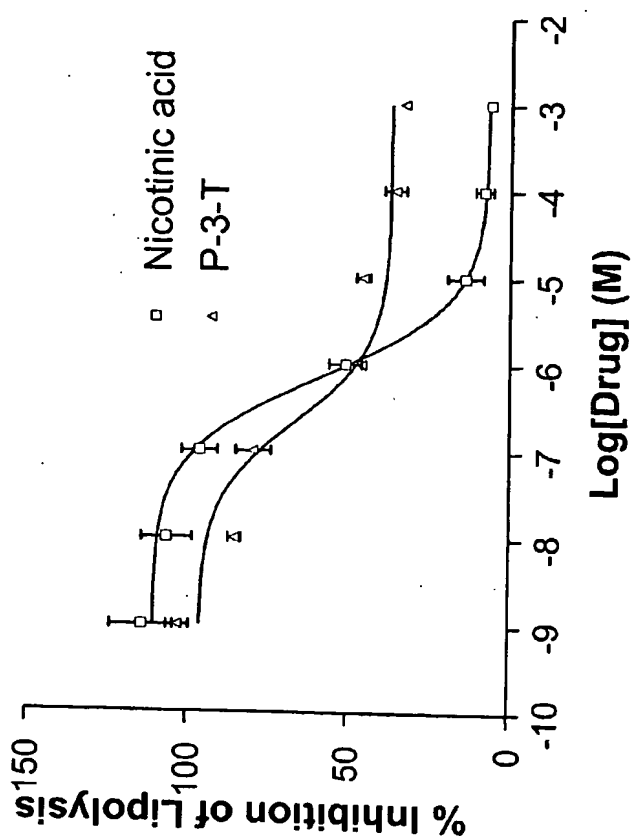
**Figure 9A**  
**Nicotinic acid and related compounds inhibit**  
**isoproterenol induced lipolysis in rat epididmal fat**  
**derived adipocytes**



**Figure 9B**  
**Nicotinic acid dose-dependent inhibition of**  
**isoproterenol induced-lipolysis in rat, epididimal fat**  
**derived adipocytes**



**Figure 10**  
**Dose-dependent inhibition of isoproterenol induced-lipolysis in human, subcutaneous-derived, primary adipocytes *via* nicotinic acid and P-3-T**



59.US1.ST25  
SEQUENCE LISTING

<110> Semple, Graeme  
Gharbaoui, Tawfik  
Shin, Young-Jun  
Decaire, Marc  
Skinner, Philip  
Averbuj, Claudia L

<120> PYRAZOLE DERIVATIVES AND METHODS OF PROPHYLAXIS OR TREATMENT OF  
METABOLIC-RELATED DISORDERS THEREOF

<130> 59 US1 PRO

<160> 6

<170> PatentIn version 3.2

<210> 1  
<211> 1092  
<212> DNA  
<213> Homo sapien

<400> 1  
atgaatcggc accatctgca ggatcacttt ctggaaatag acaagaagaa ctgctgtgtg 60  
ttccgagatg acttcattgt caaggtgttg ccgccggtgt tggggctgga gtttatcttc 120  
gggcttcttg gcaatggcct tgcctgttg attttctgtt tccacctcaa gtcctggaaa 180  
tccagccgga ttttctgtt caacctggca gtggctgact ttctactgat catctgcctg 240  
cccttctga tggacaacta tgtgaggcgt tgggactgga agtttgggga catcccttgc 300  
cggctgatgc tcttcattgt ggctatgaac cgccagggca gcatcatctt cctcacggtg 360  
gtggcggtag acaggtatctt ccgggtggtc catccccacc acgccctgaa caagatctcc 420  
aatcggacag cagccatcat ctcttgccct ctgtggggca tcactattgg cctgacagtc 480  
cacctcctga agaagaagat gccgatccag aatggcggtg caaatttgtg cagcagcttc 540  
agcatctgcc ataccttcca gtggcacgaa gccatgttcc tcctggagtt ctctctgccc 600  
ctgggcatca tcctgttctg ctgagccaga attatctgga gcctgcggca gagacaaatg 660  
gaccggcatg ccaagatcaa gagagccatc acctcatca tgggtggtggc catcgtcttt 720  
gtcatctgct tccttcccag cgtggttgtg cggatccgca tcttctggct cctgcacact 780  
tcgggcacgc agaattgtga agtgaccgc tcggtggacc tggcgttctt tatcactctc 840  
agcttcacct acatgaacag catgctggac cccgtggtgt actacttctc cagcccatcc 900  
tttcccaact tcttctccac ttgatcaac cgctgcctcc agaggaagat gacaggtgag 960  
ccagataata accgcagcac gagcgtcgag ctacacgggg accccaacaa aaccagaggc 1020  
gtccagagg cgtaatggc caactccggt gagccatgga gccctctta tctgggcca 1080  
acctctctt aa 1092

59.US1.ST25

<210> 2  
 <211> 363  
 <212> PRT  
 <213> Homo sapien

<400> 2

Met Asn Arg His His Leu Gln Asp His Phe Leu Glu Ile Asp Lys Lys  
 1 5 10 15  
 Asn Cys Cys Val Phe Arg Asp Asp Phe Ile Val Lys Val Leu Pro Pro  
 20 25 30  
 Val Leu Gly Leu Glu Phe Ile Phe Gly Leu Leu Gly Asn Gly Leu Ala  
 35 40 45  
 Leu Trp Ile Phe Cys Phe His Leu Lys Ser Trp Lys Ser Ser Arg Ile  
 50 55 60  
 Phe Leu Phe Asn Leu Ala Val Ala Asp Phe Leu Leu Ile Ile Cys Leu  
 65 70 75 80  
 Pro Phe Leu Met Asp Asn Tyr Val Arg Arg Trp Asp Trp Lys Phe Gly  
 85 90 95  
 Asp Ile Pro Cys Arg Leu Met Leu Phe Met Leu Ala Met Asn Arg Gln  
 100 105 110  
 Gly Ser Ile Ile Phe Leu Thr Val Val Ala Val Asp Arg Tyr Phe Arg  
 115 120 125  
 Val Val His Pro His His Ala Leu Asn Lys Ile Ser Asn Arg Thr Ala  
 130 135 140  
 Ala Ile Ile Ser Cys Leu Leu Trp Gly Ile Thr Ile Gly Leu Thr Val  
 145 150 155 160  
 His Leu Leu Lys Lys Lys Met Pro Ile Gln Asn Gly Gly Ala Asn Leu  
 165 170 175  
 Cys Ser Ser Phe Ser Ile Cys His Thr Phe Gln Trp His Glu Ala Met  
 180 185 190  
 Phe Leu Leu Glu Phe Phe Leu Pro Leu Gly Ile Ile Leu Phe Cys Ser  
 195 200 205  
 Ala Arg Ile Ile Trp Ser Leu Arg Gln Arg Gln Met Asp Arg His Ala  
 210 215 220

59.US1.ST25  
 Lys Ile Lys Arg Ala Ile Thr Phe Ile Met Val Val Ala Ile Val Phe  
 225 230 235 240

Val Ile Cys Phe Leu Pro Ser Val Val Val Arg Ile Arg Ile Phe Trp  
 245 250 255

Leu Leu His Thr Ser Gly Thr Gln Asn Cys Glu Val Tyr Arg Ser Val  
 260 265 270

Asp Leu Ala Phe Phe Ile Thr Leu Ser Phe Thr Tyr Met Asn Ser Met  
 275 280 285

Leu Asp Pro Val Val Tyr Tyr Phe Ser Ser Pro Ser Phe Pro Asn Phe  
 290 295 300

Phe Ser Thr Leu Ile Asn Arg Cys Leu Gln Arg Lys Met Thr Gly Glu  
 305 310 315 320

Pro Asp Asn Asn Arg Ser Thr Ser Val Glu Leu Thr Gly Asp Pro Asn  
 325 330 335

Lys Thr Arg Gly Ala Pro Glu Ala Leu Met Ala Asn Ser Gly Glu Pro  
 340 345 350

Trp Ser Pro Ser Tyr Leu Gly Pro Thr Ser Pro  
 355 360

<210> 3  
 <211> 22  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Novel Sequence

<400> 3  
 gctggagcat tcactaggcg ag

22

<210> 4  
 <211> 24  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Novel Sequence

<400> 4  
 agatcctggg tcttggtgac aatg

24

<210> 5  
 <211> 27  
 <212> DNA

59.US1.ST25

&lt;213&gt; Artificial

&lt;220&gt;

&lt;223&gt; Novel Sequence

&lt;400&gt; 5

ctgatggaca actatgtgag gcgttgg

27

&lt;210&gt; 6

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial

&lt;220&gt;

&lt;223&gt; Novel Sequence

&lt;400&gt; 6

gctgaagctg ctgcacaaat ttgcacc

27

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/018389

International filing date: 10 June 2004 (10.06.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/478,664  
Filing date: 13 June 2003 (13.06.2003)

Date of receipt at the International Bureau: 22 September 2004 (22.09.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**